THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

VOL. LXXI—No. 3

Issued February 1, 1925

BALTIMORE, U. S. A.

1925

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

Made in United States of America

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 71 FEBRUARY 1, 1925 No. 3

HISTAMINE AS A CONSTITUENT OF SECRETIN PREPARATIONS

ELOISE PARSONS

From the Hull Laboratories of Physiological Chemistry and Pharmacology

Received for publication October 9, 1924

Secretin action is found in a great many extracts and tissues. Acid extracts of duodenum, jejunum and part of the ileum, according to Bayliss and Starling (1), and confirmed by many others, have a very great secretagogue action on the pancreas, when injected into an animal. Acid maceration of mesenteric ganglion of the rat causes secretion from the pancreas, according to Camus (2). The presence of secretin in aqueous extracts of liver, thyroid and thymus, as reported by Rodgers, Rahe, Fawcett and Hackett (3), is open to criticism in that the effect produced may have been secondary to the action of a primary gastric stimulation. Luckhardt, Henn and Palmer (4) show that secretin prepared from the gastric mucosa is as effective as secretin from the duodenal mucosa in producing a secretion from the pancreas. Witte's peptone in some cases is reported to cause a secretion of pancreatic juice. Van Eweyk and Tannenbaum (5) report that casein or egg white digests have no effect until they are heated to 280°C., when they show good secretagogue action. Amino acids, according to Sweitzer (6), have no secretagogue action, but van Eweyk and Tannenbaum find that of all the amino acids, histidine alone when heated to 280°C. gives a substance which, when injected, causes the pancreas to secrete. Secretin preparations, however, heated to 280°C. lose their activity. Van Eweyk and Tannenbaum, also, confirm the finding of Bickel (7) that there is a substance in spinach which has a marked action on the flow of pancreatic juice. This spinach secretin they claim to be free from depressor action.

Voegtlin and Meyers (9) consider the antineuritic vitamine to have secretagogue action, but Anrep and Drummond (10) show that it is not identical with secretin. Uhlmann (11) found that the commercial vitamine "orypan" stimulated pancreatic secretions when injected intra-

venously, subcutaneously or by oral administration into a rabbit. He also finds the activity distributed in rice polishings, spinach, nettles, meat, oats, yeast, clover and cabbage. Popielski (12) considers that all these substances have in common "vasodilatin" which acts to cause a lowering of the blood pressure, a decrease in the coagulability of the blood, a flow of gastric and pancreatic juices, and of saliva, the general similarity of the action being due to the probable splitting of the substance with the liberation and the subsequent action of vasodilatin, which, he suggests, is in all probability due to an imidazol derivative, possibly histamine or a "histamine-like" substance.

The fact that secretin activity is found in so many different tissues suggests that the action is due either to a variety of substances which are rather widely distributed, or if it is due to a single substance, this must be a constituent of a great variety of materials.

Abel and Kubota (13) were of the opinion that histamine is a rather widely distributed substance, everywhere the same, which may have its origin in various tissues, and which makes its appearance wherever protoplasm is killed. In other words, it arises wherever a true protein is even partially disrupted by enzymes, acids or other hydrolytic agents. They obtained histamine from hypophysis, Witte's peptone, ereptone, casein (Kahlbaum) and edestin by acid hydrolysis, as well as in an acid extract of gastric mucosa and duodenal mucosa. The substance which they isolated in each case formed a picrate which melted at 236 to 237°C., gave a strong Pauly reaction, was precipitated by phosphotungstic acid and by mercuric chloride. It yielded a crystalline gold chloride and platinic chloride. As a free base, it is soluble in water, alcohol and hot chloroform, but insoluble in dry ether. These are the properties of histamine; hence their substance was identical with histamine in crystalline form, melting point and solubility. Moreover, the physiological behavior of the two showed their substance to be identical with histamine in the lowering of the blood pressure in a cat, and the contraction of the virgin guinea pig uterus.

Much of the work of Abel and Kubota has been disproved by the determination of histamine by the more accurate method devised by Koessler and Hanke (15). These workers show that hypophysis which has not been acted upon by bacteria contains no histamine. Peptone prepared under sterile conditions contains no histamine, but is capable of producing a typical peptone shock. An analysis of pure casein hydrolyzed by acids, shows no histamine, but the pharmacological findings verify those of Abel and Kubota, in that there is a fall in the blood pressure when it is injected intravenously into an animal. The conclusion is, that there is a substance present in the peptone and hydrolyzed casein, which behaves similarly to histamine, but which is not identical with histamine.

Barger and Dale (14) obtained from the mucosa of the small intestines of an ox, a picrate quite similar to histamine picrate, the melting point of which was 232°C. It gave the Pauly reaction for imidazol and physiologically caused a fall in blood pressure and a flow of pancreatic juice. They state, however, that the substance has less power to produce pancreatic juice than a dose of secretin which depresses to the same amount.

The development of the accurate chemical method of Koessler and Hanke (15) for the determination of histamine in protein and protein containing matter, makes possible the accurate determination of histamine in secretin solutions. The method is specific for histamine; other substances which are similar are eliminated in the process of the analysis. When a protein containing extract is analyzed by this method and found to contain histamine, then, if the activity is similar qualitatively and quantitatively to histamine, that activity may be said to be due to histamine, provided the quantity present is comparable with an amount of pure histamine which produces the same effects.

Secretin, according to one prevailing opinion, owes its activity to histamine. It was thought that by determining the histamine present in a secretin preparation, and by comparing these results with the determinations on secretin preparations to which known but reasonable amounts of histamine had been added, one might throw some light on the claimed identity of histamine and secretin.

EXPERIMENTAL: *Preparation of secretin.* The duodenum and part of the jejunum were taken from dogs, killed just previously by bleeding. This material was washed thoroughly in cold tap water, dried between towels, the mucosa scraped off, ground and weighed. It was then dropped into hot 0.4 per cent hydrochloric acid solution, using 4 cc. for every gram of scrapings. The mixture was stirred while being brought to a temperature of 90 to 95°C. and allowed to stand over night. The next morning it was again brought to 95°C. and filtered. The filtrate was concentrated under diminished pressure, until 1 cc. was equivalent to 4 grams of the original scrapings. The concentrated secretin was neutralized with Na_2CO_3 and filtered, the neutral filtrate being used for the experiments. In the preparation, it was less than 30 minutes from the death of the animal till the material was in the hot acid.

Histamine. Purified histamine dihydrochloride was used. This was a synthetic product, which contained 94.8 per cent histamine $(HCl)_2$ and 5.2 per cent of NaCl and H_2O together. A stock solution was made by dissolving 2.1097 grams in water, diluting to exactly 200 cc. From this a solution was made by diluting 1 cc. of the stock solution to 100 cc. Each cubic centimeter contained 0.0001 gram histamine dichloride.

Estimation of histamine. The method of Koessler and Hanke (15b) was followed exactly. The material was in the liquid form, so that the

excessive humus formation noted by Gerard (20) in the acid hydrolysis was minimized.

Briefly, the method consists of *1*, hydrolysis of the material in 20 per cent HCl for 30 hours; *2*, removal of the hydrochloric acid by distillation in vacuo; *3*, removal of the ammonia from alcoholic solution in which there is an excess of lime, by distillation in vacuo; *4*, removal of the humin by filtration; *5*, precipitation of the hexone bases from an acid solution by phosphotungstic acid; *6*, decomposition of the precipitate by saturated barium hydroxide; *7*, extraction of the histamine with amyl alcohol from a strongly alkaline solution; *8*, recovery from the amyl alcohol by an acid solution and neutralization of the acid with barium hydroxide; *9*, precipitation of the histamine by silver nitrate and barium hydroxide; *10*, final purification of the histamine by means of chloroform.

The histamine fraction thus obtained is estimated colorimetrically by a modification of the Pauly reaction in which the amounts of reagents, sodium nitrite and sulphanilic acid are accurately measured, as well as the time during which they react. The alkalinity is carefully controlled also. The color is compared with a standard which is a mixture of congo red and methyl orange and which matches the color formed in the reaction of histamine perfectly.

In the preliminary work with this method, it was found that the decomposition of the phosphotungstate formed by the barium hydroxide required considerable attention. Unless the precipitate was triturated with $Ba(OH)_2$ quite thoroughly in a mortar and unless the digestion was over a long period of time, some of the undecomposed precipitate remained. Since this is the first step in the analysis in which the secretagogue activity disappears, it was thought that the Van Slyke acid decomposition method might eliminate this loss of secretagogue reaction in secretin preparations. The analysis for histamine using both methods shows that the acid decomposition of the precipitate is as efficient as the alkaline for histamine, but the physiological tests for secretagogue action of secretin were quite negative by either method.

The report of a trace of color means that there was a color formed, but it was impossible to estimate the amount. The samples which were concentrated to 1 cc. gave a color reading. The color which appeared, but which was not on the scale of the colorimeter appeared at the same time after the addition of the reagents as the color for histamine and was the same shade as colors formed by histamine. The color readings are corrected for the colorimeter used and also by the factor for the reagents given by Koessler and Hanke. The equivalent amounts of histamine are taken from the tables prepared by the same authors.

Analysis. Five different secretin preparations were analyzed for histamine.

Experiment 1. Secretin "6" was a very active preparation, 1 cc. injected intravenously caused a fall in blood pressure of 60 mm. Hg and 36 drops of pancreatic juice in 10 minutes.

In the preparation of secretin, a gummy residue formed when the material was concentrated. This was usually filtered off and disregarded, but with this particular preparation it was saved and the histamine content determined. When the preparation was neutralized there always formed a protein precipitate which was filtered off. This was also saved and analyzed.

Two 100 cc. portions of the concentrated secretin were analyzed for histamine. Two other 100 cc. portions were analyzed, to each of which 1 mgm. of pure histamine dihydrochloride had been added.

Quantitative amount of histamine in secretin "6". One hundred cubic centimeters secretin during the analysis process was concentrated to 5 cc. One cubic centimeter of this gave a corrected reading of 35.4 on the colorimeter, which is calculated from tables to contain 0.0000474 gram of histamine. This makes 0.0002379 gram in the total 100 cc. analyzed. Duplicate analysis confirmed the result.

One hundred cubic centimeters of the same secretin preparation to which 0.001 gram pure histamine was added, was concentrated in the process of analysis to 25 cc., 0.5 cc. of which gave a colorimetric reading of 20.1, equivalent to 0.000025 gram histamine or 0.00125 in the total sample.

There was merely a trace of color from the gummy residue formed in the process of concentration.

There was only a trace of color from the precipitate formed when the secretin was neutralized.

The mean from the analysis above is:

0.00125 gram	histamine from 100 cc. secretin plus 0.001 gram pure histamine $(HCL)_2$
0.000234 gram	histamine from 100 cc. secretin above
0.001016 =	histamine recovered = 101.6 per cent

The amount of histamine present in 100 cc. secretin was 0.23 mgm.; the amount in 1 cc. which had strong physiological action when injected intravenously, was 0.0023 mgm.

The recovery of histamine shows that the technique and the method are satisfactory. Negative findings on the material which is being disregarded shows that no histamine is being discarded when the secretin is being prepared from the intestinal mucosa.

Experiment 2. Secretin "7" was a very active preparation which was obtained from the Department of Physiology. One cubic centimeter when injected intravenously into a dog caused a fall in blood pressure of 55 mgm. of mercury and the flow of 16 drops of pancreatic juice in 10 minutes.

A 10 cc. portion was analyzed and the final solution concentrated to 10 cc. One cubic centimeter of this gave a color which appeared at its maximum intensity 4 to 5 minutes after the addition of the reagents, and which was the same shade of color as obtained when histamine was present, but the color was not intense enough to be on the scale of the colorimeter used.

The conclusion is that there was a *very* small amount of histamine present in 10 cc.

Experiment 3. Secretin "8" was a preparation from the Department of Physiology which had active secretagogue action as compared with a small depressor action. There was a fall in blood pressure of 30 mm. Hg when 4 cc. were injected intravenously, but a flow of 24 drops of pancreatic juice, in 10 minutes.

A 10 cc. portion of this was analyzed and the final solution concentrated to 10 cc. One cubic centimeter of this failed to give a color intense enough to be read on the colorimeter.

Experiment 4. In the preparation of secretin "9", the intestinal mucosa was digested in hot alcohol which contained 0.4 per cent HCl, the alcoholic extract being evaporated and the further preparation being as usual. In this preparation, the mucosa was in the hot alcohol about 15 minutes after the death of the animal.

Duplicate analyses were made on the secretin, using a 25 cc. sample in each case, and duplicate analyses were made on 25 cc. samples to each of which 0.001 gram histamine $(HCl)_2$ had been added.

The 25 cc. portion analyzed concentrated to 5 cc. gave just a trace of color formation. Concentration to 1 cc. or 0.5 cc. would have given a color which would have been on the scale of the colorimeter.

Another 25 cc. portion to which 0.001 gram histamine had been added was carried through similar analysis. Five-tenths cubic centimeter gave a corrected color reading of 15.8 which is equivalent to 0.000021 gram of histamine or 0.00105 in the total 25 cc. sample of secretin. Duplicate analysis gave 0.00110 gram histamine in total sample. A mean of the two is 0.001075 gram histamine.

A check analysis was made on 0.001 gram pure histamine in 10 cc. water. Five-tenths cubic centimeter of this gave a color reading of 38.0, equivalent to 0.00005 gram of histamine or 0.001 gram in the 10 cc. sample.

If the recovery of histamine is taken as 100 per cent as found, then the amount of histamine in 25 cc. of the secretin sample, according to 3, 4 and 5, is 0.000075 gram or 0.30 mgm. in 100 cc. of preparation "9". This is nearly of the same order as found in no. 6 which was 0.234 mgm. in 100 cc.

Experiment 5. Preparation 10 was made according to the usual method.

Only 10 cc. samples were used for the analyses, and only 0.1 mgm. histamine added for the control experiments.

In this analysis the phosphotungstate precipitate was decomposed by the Van Slyke acid method in one sample and by the usual alkaline method in the other. The results show that histamine could be recovered equally well by either method.

Ten cubic centimeters secretin were analyzed by the same method which has been used in all the samples analyzed, in which the phosphotungstate precipitate is decomposed by alkali. Concentrated to 1 cc., 0.5 of which gave a corrected colorimetric reading of 6.7, which is equivalent to 0.000009 gram of histamine or 0.000018 gram in the total sample.

Ten cubic centimeters of the same secretin preparation, in which the phosphotungstate precipitate was digested by acid according to the method of Van Slyke gave a value of 0.000016 gram histamine (HCl_2) in the total sample.

Ten cubic centimeters of the same secretin preparation to which 0.0001 gram of pure histamine had been added were analyzed, using alkaline digestion of the phosphotungstate precipitate. Five-tenths cubic centimeter gave a colorimeteric reading of 4.5, equivalent to 0.000006 gram histamine or 0.00012 gram in the total sample.

A duplicate of the sample analyzed above was carried through the process with acid digestion of the phosphotungstate precipitate. Five-tenths cubic centimeter gave a corrected colorimetric reading of 4.3, equivalent to 0.0000055 gram histamine, or 0.00012 in the total sample.

One-hundredth gram histamine analyzed with the usual alkaline digestion of the phosphotungstate precipitate gave a recovery of 0.0096 gram of histamine.

One-hundredth gram histamine analyzed using the acid digestion of phosphotungstate precipitate, showed a recovery of 0.0092 gram histamine.

In this sample of secretin.

0.00012 gram histamine = amount in 10 cc. secretin plus 0.0001 gram pure histamine

0.000017 gram histamine = amount in 10 cc. secretin above

0.000103 = 103 per cent = histamine recovered

The presence of 0.000017 gram in 10 cc. is equivalent to 0.00017 in 100 cc. which compares favorably with the 0.00023 gram found in secretin preparation "6", and the calculated amount, 0.0002 in secretin preparation "9".

The two methods of decomposition of the phosphotungstate precipitate were used in order to see if the loss of the secretagogue action of secretin could be prevented by the acid treatment. In each case all the depressor substance was precipitated by the phosphotungstic acid, and was re-

covered equally well by acid and by alkaline decomposition. The secretory activity could not be detected in the precipitate, nor did it remain in the filtrate. Comparable evaporations were made in every case so that the amount injected was comparable with the original secretin.

SUMMARY OF RESULTS. There is a very small amount of histamine present in secretin preparations.

That this amount of histamine is not enough to be of physiological importance is shown in the experiments which follow.

TABLE 1

Summary of quantitative estimation of histamine in secretin preparation

PREPARATION NUMBER	HISTAMINE FOUND	HISTAMINE IN 1 CC.
	gram	*gram*
6	0.000234 (in 100 cc.)	0.0000023
7	Trace (in 10 cc.)	
8	Trace (in 10 cc.)	
9	0.000075 (in 25 cc.)	0.000003
10	0.000017 (in 10 cc.)	0.0000017

PHYSIOLOGICAL ACTIVITY. In the same preparations which were analyzed chemically for the presence of histamine, the physiological action was followed. The action of the original preparation when injected into animals was compared with the actions of the different fractions obtained in the process of analysis, and finally with the final solution upon which the colorimetric estimation of histamine was made.

Preparation of animals for physiological experiments. Healthy dogs were used. An hour before the experiment the animal was given 0.25 gram barbital per kilo body weight. This was suspended in 50 cc. of water and put into solution with as little Na_2CO_3 as necessary. It was administered to the dog by stomach tube. The dog goes to sleep, and in most instances is so analgesic at the end of 30 to 45 minutes, that the operative procedure can be done without further anesthesia. In some dogs it is necessary to administer a small amount of ether at the time of the incision, but no further administration is necessary.

It has been found that under this anesthesia, the blood pressure maintains a high level, the respiration is slow and regular, and the secretory functions respond normally. This eliminates the variations in the blood pressure which are constantly present under ether anesthesia and the inhibition of gastric and pancreatic secretion so often observed under ether.

The animal was prepared by ligature about the pylorus, and a cannula in the pancreatic duct. Tracings were made registering the respiration,

blood pressure from the carotid artery, and the number of drops of pancreatic juice. Injections were made into the saphenous vein.

Table 2 shows the results obtained from secretin preparation "6".

Discussion. The results show that secretin "6" is a very active preparation, causing a fall in blood pressure of 68 mm. Hg and 36 drops of pancreatic juice in 17 minutes. After this has been digested with 20 per cent HCl for 30 hours, the fall in blood pressure is only 46 mm. Hg and there are only 3 drops of pancreatic juice in 10 minutes. When the material is treated with lime in an alcoholic solution to remove ammonia, there is no further loss in depressor or secretagogue action.

TABLE 2

Physiological activity of fractions obtained in analysis of secretin no. "6" for histamine

INJECTIONS	BLOOD PRESSURE					PANCREATIC SECRETION		
	Normal	Lowest reached	Fall	Time for return		Latent period	Drops juice	Time
				minutes	*seconds*	*seconds*		*minutes*
1 cc. concentrated secretin "6"	116	48	68	17	2	93	36	17
1 cc. after acid hydrolysis for 30 hours	110	64	46	2	15	50	3	10
1 cc. after removal of ammonia	90	42	48	12		50	3	10
1 cc. decomposed P. T. A. precipitate	100	50	50	2				
1 cc. residue from above	108	No action						
1 cc. after extraction with amyl alcohol	96	50	46	2	15			
1 cc. residue	100	No action						
1 cc. after Ag precipitation final solution	106	78	28	0	27		Histamine content 0.0023 mgm. per cubic centimeter	

After precipitation with phosphotungstic acid and decomposition of the precipitate, there remains a depressor action of the same order, but there is no secretagogue effect. Acid decomposition produces the same results, as stated before. In the solution from which the hexone bases were precipitated, there was neither depressor action nor secretagogue action. Somehow, in the treatment, the secretagogue material was destroyed or lost. Amyl alcohol removes the depressor substance from an alkaline solution, but in this process the secretagogue action is also lost. The final solution has a depressor effect on the blood pressure causing it to fall about one-half as much as the original secretin, but there is no secretagogue action remaining.

The results in table 3 are to be compared with those obtained from a secretin preparation to which a known amount of histamine was added. The fractions are similar to those in table 2.

The results above are to be compared with those obtained with histamine alone, when it is carried through the same process for quantitative analysis.

Injection of 0.0001 gram of histamine caused a fall in blood pressure from 104 mm. Hg to 40, a drop of 64 mm. Hg which did not return for 5 minutes 15 seconds. After a latent period of 2 minutes 34 seconds there were 6 drops of pancreatic juice which flowed in a period of 10 minutes.

After the removal of the ammonia, which occurs in the process of analysis, the histamine solution was injected and the effects noted. The blood pressure fell from 102 to 34 mm. Hg and returned to normal after a

TABLE 3

Physiological activity of secretin fractions to which histamine has been added

INJECTION	BLOOD PRESSURE					PANCREATIC JUICE		
	Normal	Lowest reached	Greatest fall	Time for return		Latent period	Drops juice	Time
				minutes	*seconds*	*seconds*		*minutes*
1 cc. secretin with 0.1 mgm. histamine	106	32	72	13	15	45	43	10
1 cc. after removal of ammonia	104	34	70	7	30	45	8	10
1 cc. after amyl alcohol treatment	106	40	60	5		92	5	10
1 cc. after Ag precipitation	100	38	62	2	30	90	4	10

period of 6 minutes. There was a flow of 6 drops of pancreatic juice after a latent period of 2 minutes 30 seconds.

When the histamine-containing material was injected after precipitation with phosphotungstic acid, the blood pressure fell from 108 mm. Hg to 48, a fall of 60 mm., which returned in 3 minutes 50 seconds. There were seven drops of pancreatic juice which flowed for a period of ten minutes following a latent period of 2 minutes 40 seconds.

The treatment with amyl alcohol had no deleterious effect on the physiological action. After treatment with amyl alcohol the histamine-containing solution injected caused a fall in blood pressure from 106 to 44 mm. Hg, a lowering of 62 mm. which returned to normal after 4 minutes 30 seconds. There were 5 drops of pancreatic juice which flowed in ten minutes following a latent period of 2 minutes 35 seconds.

The final precipitation with silver left a solution which caused a fall

in blood pressure from 100 to 44 mm. Hg. This drop of 56 mm. was recovered from in one minute 10 seconds. The injection also caused a flow of 5 drops of pancreatic juice after a latent period of 2 minutes 40 seconds.

Discussion. Histamine causes a flow in pancreatic juice, but only about one-sixth as much as a secretin preparation which depresses to the same amount. The secretagogue action of histamine is as strong in the final solution as in the original, a flow of 6 drops being observed in the original, as compared with 5 drops in the final solution, in the same period of time. In the secretin solution to which histamine had been added (table 3) the result of the injection of the two together seems to be the additive effect of their individual actions. After the precipitation of the hexone bases by phosphotungstic acid, and the treatment of the alkaline solution with amyl alcohol, the effect due to the secretin disappears, and the results are similar to those obtained with histamine alone.

Similar results were obtained in the other preparations of histamine analyzed.

SUMMARY OF RESULTS. 1. Secretin loses its ability to cause the pancreas to secrete during the process by which it is analyzed for histamine.

2. Histamine, which has a lower, but nevertheless decided secretagogue action, loses none of it by the same process.

3. Secretin, to which histamine has been added, when thus treated, loses the activity due to the secretin, but retains the activity due to the histamine.

4. The depressor action is lessened after the analysis in the case of secretin, but is still present. It is but slightly lessened in histamine.

INSTABILITY OF SECRETIN. It is not surprising that the results of the vigorous chemical treatment involved in the analysis for histamine leave a material which shows no secretagogue activity, in view of the instability which many secretin preparations exhibit. Some preparations of secretin in acid or neutral solution retain their activity for months, others lose theirs in a few days, even though they are kept under the same conditions, apparently.

The results (table 4) are taken as being illustrative of the instability. A sample of each preparation of secretin was kept in a sealed tube which had been sterilized by bringing it to a boil on three successive days. The stock solution was covered with a layer of toluol and kept in the ice box.

All of the preparations were made according to the process described; the initial activity of each was indicative of an active preparation. There is no uniformity of results; O_1 and S_2 are active even though they are over a year old, and have had no special treatment to prevent destruction.

On the other hand A_2, M, L_4, have lost their activity no matter whether kept in a sealed tube or under toluol in the ice box.

Instability in acid solution. In all the preparations 0.4 per cent HCl was used, and in each case the secretin was made neutral to litmus before it was injected, to test its activity.

The stability of secretin in acid solutions. Secretin C had such an activity that when 4 cc. were injected there was a flow of 22 drops of pancreatic juice in 10 minutes.

TABLE 4

Instability of secretin on standing

PREPARATION	DATE PREPARED	DATE TESTED	ACTIVITY (DROPS PANCREATIC JUICE IN 10 MINUTES)	REMARKS
A_2	November 17, 1921	November 23, 1921	15	Kept in sealed tube
		November 27	1	
O_1	February 11, 1922	February 13, 1922	17	Kept in ice box
		August 15, 1923	11	Kept in ice box
		July 2, 1923	7	
		July 2, 1923	9	Kept in sealed tube
M_1	March 3, 1922	March 4, 1922	22	Kept in ice box
		March 17, 1922	2	Kept in ice box
S_2	March 8, 1922	March 10, 1922	18	Kept in ice box
		August 15, 1922	15	Kept in ice box
		July 2, 1923	10	Kept in ice box
L_4	April 24, 1922	April 24, 1922	23	
		August 15, 1922	2	Kept in sealed tube
M_3	August 8, 1922	August 15, 1922	30	
		July 2, 1923	18	Kept in sealed tube

The same secretin evaporated to dryness on the steam bath, made up to volume again with water, when injected causes only a small flow of 7 drops of juice in a 10-minute interval.

Four cubic centimeters of secretin D when injected stimulated a flow of 40 drops of pancreatic juice in 10 minutes.

After concentrating in vacuo, then making up to the original volume, the injection of 4 cc. instigated a flow of 44 drops of pancreatic juice in 10 minutes.

When the secretin was heated on the steam bath with a reflux (condenser) for 6 hours, there was a loss of activity. The injection of 4 cc. caused a flow of 7 drops in 20 minutes.

Similar results were obtained with four other preparations of secretin. Evaporation of a secretin solution in acid, out in the air, causes it to lose two-thirds of its activity. Boiling with a reflux condenser causes it to lose about two-thirds of its activity also. Concentration in vacuo at 50 to 60°C. does not destroy the activity of secretin, whether it is concentrated with 0.4 HCl or whether it is neutralized before concentration.

Instability in alkaline solutions. The most active secretin preparations are those in which the acidity is kept about 0.4 per cent HCl, and in which the material at no time becomes alkaline, or if so, for only a very short time. Boiling with alkali even with dilute alkali, completely destroys the activity. A secretin solution which stands in a strong NaOH solution over night loses its activity.

A secretin preparation which is made only slightly alkaline to litmus, does not lose its activity entirely but to some extent. The alkalinity

TABLE 5

The instability of secretin in alkaline solutions

PREPARATION	ACTIVITY (DROPS PANCREATIC JUICE)	TREATMENT
Secretin Y	30 (in 15 minutes)	4 cc. of the unconcentrated secretin was injected intravenously
	1 (in 30 minutes)	After boiling with 1 per cent NaOH 6 hours. Neutralized before injection
	0 (in 30 minutes)	After standing in 29 per cent NaOH over night
	12 (in 15 minutes)	After being N/10 alkaline for 6 hours
	15 (in 15 minutes)	After treatment with lime in 50 per cent alcohol, concentrated in vacuo

present when lime in excess is added to an acid solution in 50 per cent alcohol lowers the activity but does not destroy it. The concentration of alcohol (2 grams NaOH to 10 cc.) in the solution from which histamine is extracted with amyl alcohol, is sufficient to destroy the secretagogue action of a secretin solution.

Typical results are tabulated in table 5.

In each case 50 cc. of the secretin was treated as described, and the amount injected was concentrated so that the original volume remained. The neutralized solutions were injected intravenously.

Instability of the phosphotungstic acid precipitate of secretin. The loss of the secretagogue action of secretin preparations has been brought out in the analysis of the preparation for histamine. The physiological assay of the fractions showed that this loss occurred after the solution was treated with phosphotungstic acid, and that the action could not be recovered from the precipitate, nor from the filtrate.

A secretin preparation which was neutral after having been concentrated in vacuo was made slightly acid and precipitated with phosphotungstic acid. The precipitate was decomposed by HCl and the phosphotungstic acid removed by an immiscible solvent of equal parts of amyl alcohol and ether.

Similar results were obtained from two other equally active preparations of secretin.

Instability in amyl alcohol. Secretin cannot be extracted from an alkaline solution by amyl alcohol. When an extract of duodenal mucosa is treated with amyl alcohol, the secretagogue action is neither extractable from the alcohol by acid treatment, nor does it remain in the alkaline water phase.

Extractions with amyl alcohol were made of secretin preparations, the alkalinity of which was varied, and the destructive effect of the alkali alone was noted.

TABLE 6

The instability of secretin precipitated by phosphotungstic acid

PREPARATION	ACTIVITY (DROPS PANCREATIC JUICE)	TREATMENT
Secretin J	20 (in 10 minutes)	1 cc. of concentrated secretin
	1 (in 10 minutes)	P. T. A. precipitate decomposed by acid, concentrated, neutralized
	0 (in 30 minutes)	Filtrate from the precipitate concentrated, neutralized

Typical results on a number of different preparations of secretin, having been variously treated, are as follows:

When 2 cc. of concentrated secretin were injected intravenously there was a fall of blood pressure from 110 to 40 mm. of mercury and a flow of 24 drops of pancreatic juice in ten minutes.

A portion of the same secretin which had been made alkaline with 0.4 per cent NaOH, kept alkaline for 4 hours, then neutralized and concentrated to the original volume, when injected showed a small decrease in activity. There was a fall in blood pressure from 100 to 48 mm. of mercury, and a flow of 15 drops of pancreatic juice in 15 minutes.

Some of the alkalinized secretin preparation was extracted with amyl alcohol, and recovered from the amyl alcohol with 0.4 per cent HCl, then concentrated to the original volume. When injected it caused a fall in blood pressure from 100 to 60 mm. Hg, but there was no flow of pancreatic juice.

The solution remaining after the amyl alcohol extraction, caused a fall in blood pressure from 100 to 68 mm. Hg but no stimulation of pancreatic juice.

Secretin which was kept more strongly alkaline, with 1 per cent NaOH for 4 hours, then neutralized and concentrated to volume, caused a fall of blood pressure from 104 to 50 mm. Hg and a secretion of 10 drops of pancreatic juice in 10 minutes.

Amyl alcohol extraction from the 1 per cent NaOH solution of secretin, and recovery with 0.4 per cent HCl, left a solution which caused a fall in blood pressure from 98 to 52 mm. Hg, but no stimulation to pancreatic flow.

The solution remaining after the extraction caused a very slight fall in blood pressure from 98 to 80 mm. Hg and no flow of pancreatic juice.

Secretin which was made very strongly alkaline with 20 per cent NaOH for 4 hours, then neutralized and concentrated to volume, caused a decrease in blood pressure from 106 to 52 mm. Hg, but there was no stimulation to pancreatic flow.

This 20 per cent alkaline solution extracted with amyl alcohol, recovered with 0.4 per cent HCl neutralized, concentrated and injected produced a fall in blood pressure from 102 to 50 mm. Hg, but there was no flow of pancreatic juice following.

The solution remaining after the amyl alcohol extraction, contained neither depressor substance nor secretagogue action.

The results show that alkalinity is an important factor in destroying secretagogue action. The alkalinity of 0.4 per cent NaOH, and 1 per cent NaOH did not destroy the activity but reduced it considerably, the 1 per cent NaOH more than the 0.4 per cent. The 20 per cent NaOH completely destroyed the activity.

In all instances the secretagogue activity was destroyed by amyl alcohol extraction. Amyl alcohol extracts the depressor substance from a strongly alkaline solution also.

Secretin is extracted from a solution by Fuller's earth. Secretin is taken out of a solution by treatment with Fuller's earth, and can be recovered by extraction of the earth by treatment with acid. Fuller's earth which has been saturated with NaCl, does not remove the secretin.

This indicates that secretin is a basic substance, which is taken up by the Fuller's earth. Fuller's earth here acts as does permutit in absorbing the ammonia from solutions.

Stability of secretin after freezing. Ground duodenal mucosa was frozen for 24 hours at $-18°C$. It was then chopped in the Kossel machine and the juice pressed out by a hydraulic press. This was made acid, the proteins which precipitated out were filtered off, the solution neutralized and made up to volume, so that 1 cc. represented 4 grams of the mucosa. A control secretin was made with a portion of the ground duodenum in the usual way, by heat extraction with HCl.

The action of freezing on secretin. The control secretin made in the

usual manner when injected, produced a fall in blood pressure from 120 to 54 mm. Hg, and stimulated a flow of 32 drops of pancreatic juice in 20 minutes.

Secretin prepared from ground-up frozen duodenum showed similar results, a fall of pressure from 134 to 64 mm. Hg, and 29 drops of pancreatic juice in 20 minutes.

Secretin prepared from the pressed juice exhibited about the same activity, a fall in pressure from 136 to 54 mm. Hg, and 30 drops of pancreatic juice in 20 minutes.

The secretagogue action of the secretin from the frozen mucosa and from the pressed juice is as great as the original secretin, prepared in the ordinary way. If the activity were due to intracellular substances, it should have increased when the cells were disrupted in the process of freezing.

TABLE 7

Secretin treated with Fuller's earth

INJECTION AND TREATMENT	BLOOD PRESSURE			PANCREATIC SECRETION	
	Normal	Low	Fall	Drops	Time
					minutes
1 cc. concentrated secretin	96	40	56	27	10
100 cc. concentrated secretin treated with Fuller's earth. Recovered by pressing out the juice	94	58	36	27	10
Fuller's earth remaining treated with 0.4 per cent HCl	98	50	48	20	10
Concentrated secretin treated with earth saturated with NaCl previously	100	50	50	18	10

Quantitative comparison of the physiological activity of secretin and histamine. Histamine and secretin, as ordinarily prepared, both possess depressor action and secretagogue action. Popielski attributes the two actions to "vasodilatin" which he considers a constituent of most tissue extracts and suggests the similarity to histamine.

The separation of the depressor action and the secretagogue action is a very difficult matter. Bayliss and Starling tried to prepare a secretin free from vasodilatin by extracting the depressor substance with absolute alcohol, but their protocols show that with a decrease in depressor effect, there is also a decrease in the secretory action. Matsuo (19) found that the acid which had remained in the duodenum for a short time, 5 to 10 minutes, contained a substance which when introduced intravenously caused a secretion from the pancreas without a fall in blood pressure. However, if the acid remained longer in the duodenum, 30 to 45 minutes, it contained a substance which not only caused a secretion, but also a

depression of the blood pressure. This work has been confirmed recently by Luckhardt and Grogan (16).

Depressor substances do not cause a secretion of pancreatic juice necessarily. Arthus (17) tried a variety of substances which produced a fall in blood pressure and anaphylactic shock, but found that they did not cause a secretion of the pancreas, nor augment a secretion which was normal. Preparations of duodenal mucosa retain their depressor effect after their secretagogue action has disappeared.

The experiments of previous workers have been carried out on animals under ether anesthesia, so that the results may be due to variations in the anesthesia and the asphyxia of the cells due to the anesthesia. Under the barbital anesthesia which has been used in these experiments, the blood pressure varies within such narrow limits that the depressor effects can be quantitatively measured. The secretagogue action of a histamine solution, which produces the same fall in blood pressure as a secretin preparation, can be compared with the secretagogue action of the same secretin solution.

Physiological action of histamine and secretin. The injection of 2 cc. secretin caused a fall in blood pressure of 66 mm. Hg, a drop from 96 to 30 mm. The return to normal occurred after 12 minutes. After a latent period of 75 seconds, there were 28 drops of pancreatic juice within the next 12 minutes after which the flow was normal.

The injection of 0.0001 gram of pure histamine produces a fall of 62 mm. Hg, from 90 to 28, with a return to the normal height in 6.5 minutes. Following a latent period of 100 seconds there is a flow of 4 drops of pancreatic juice in 12 minutes.

The injection of a large dose of histamine, namely, 0.00015 gram, caused a drop of 64 mm. in the blood pressure, from 100 to 36, which returned to the normal height in 6 minutes. After a period of 130 seconds, there was a flow of 6 drops of pancreatic juice during the next 12 minutes.

To show that the secretory mechanism was intact, 2 cc. of secretin used above were injected which caused a fall of 66 mm. Hg, from 98 to 32, with a return to normal in 12 minutes 60 seconds. After the injection there were 23 drops of pancreatic juice in 12 minutes.

Increasing doses of pure histamine $(HCl)_2$ were injected: 0.0002 gram caused a fall of 62 mm. Hg and a flow of 4 drops of pancreatic juice; 0.0003 gram produced a drop of 66 mm. Hg, and a secretion of 6 drops of pancreatic juice; 0.0006 gram resulted in a fall of 72 mm. in the blood pressure and a flow of 11 drops of pancreatic juice; 0.0012 gram injected produced a fall from 112 to 30 mm. Hg, a fall of 82 mm. There were only 8 drops of pancreatic juice after a latent period of 140 seconds.

Deep intramuscular injections of histamine failed to cause any fall in the blood pressure. Deep intramuscular injections of secretin likewise

produce no effect on blood pressure. Neither causes a flow of pancreatic juice. (In other experiments it has been shown that while there is no effect on the pancreatic secretion, the intramuscular injection does cause a flow of gastric juice.)

From the blood pressure tracing and the tables one can draw the following conclusions:

1. The blood pressure tracing obtained after the injection of secretin is very similar to that obtained after the injection of histamine. In each case there is a quick initial fall followed by a slight rise after which there is a secondary fall followed by a steady rise until there is a return to normal. The return to normal is more prompt after the injection of histamine than it is after secretin. Even large doses of histamine which cause very great depression return to the normal level very soon, in most cases after about 5 minutes. Secretin, which depresses to the same amount requires a longer time to return to normal.

2. The number of drops of pancreatic juice is increased with secretin much more than after an injection of histamine, 28 drops after secretin as compared with 4 to 6 drops after histamine, in the same length of time, and with the same fall in blood pressure. That is, histamine is the less efficient secretagogue, but the more powerful depressor.

3. The time from the injection of the substance to the initial drop of juice is longer with injections of histamine (the latent period in the table).

4. Very large amounts of histamine did not augment the secretion from the pancreas much more than a dose one-tenth the amount. In no case was the flow comparable to that caused by secretin.

5. Finally, histamine itself is not responsible for the "secretin" action which causes the flow of pancreatic juice, although there is a histamine-like action in the effect on the blood pressure.

Effect of introducing secretin and histamine into the duodenum. Secretin solutions were prepared as for the previous experiments, and histamine solutions were made in NaCl of the same strength as the salt content of the neutralized secretin preparations. In addition preparations of gastrin, prepared from the gastric mucosa in exactly the same way as the secretin, were used.

The dogs were under barbital anesthesia. The pancreatic duct was cannulated, the pylorus tied off, the bile duct ligated, as well as the accessory pancreatic ducts. A loop of the intestine including the duodenum and a small part of the jejunum was isolated and a cannula placed in each end.

Blood pressure tracings were made from the carotid artery. The intravenous injections were made into the saphenous vein.

In order to control all factors and to be sure that the secretory mechanism was intact, the following order of experimentation was carried out:

Outline of the experiment. 1. Control on the activity of the pancreatic mechanism by the intravenous injection of secretin of known value.

2. Determine the minimum dose of the secretin to be tested which gives similar results.

3. Determine the minimum dose of histamine which reduces the blood pressure to the same amount as the secretin.

4. Test the activity of the gastrin as to its effect on blood pressure and pancreatic secretion.

5. Wash out the intestinal loop with 50 cc. of normal salt solution.

6. Introduce 50 cc. neutral secretin into the loop and allow to remain 30 minutes, while observing the blood pressure and the rate of pancreatic secretion. Test the recovered material for its action by intravenous injection.

7. Introduce 50 cc. histamine solution into the loop, watch the effects for 30 minutes, remove and inject some of the recovered material intravenously.

8. Repeat with neutral gastrin.

9. Test the absorbent ability of the intestinal loop by the introduction of 50 cc. 0.4 per cent HCl.

10. Retest the secretory mechanism by the intravenous injection of the same secretin of known value.

Results. Three different dogs were used with three different preparations of secretin and gastrin. The results in general are very similar.

When the material was removed from the loop, more than the original 50 cc. introduced were recovered in spite of the fact that the salt content was that of a physiological solution. The loop was washed with 0.9 per cent NaCl and the washings added to the filtrate, the total volume being made up to 100 cc. Since this was twice the amount introduced, the intravenous injection made was with 2 cc., twice the volume of the original secretin solution injected. In this way all results were made comparable.

The results on one of the animals are sufficient to show the typical findings.

When 50 cc. secretin were introduced into the loop, 65 cc. were recovered at the end of 30 minutes.

When 50 cc. histamine solution were injected, 70 cc. were recovered.

After the introduction of 50 cc. gastrin for 30 minutes, 75 cc. were recovered.

Absorption of secretin from the duodenum. One cubic centimeter of secretin injected intravenously produced a fall from the normal blood pressure of 110 to 50 mm. Hg, a fall of 60 mm. The blood pressure returned to normal in 7 minutes 7 seconds. This injection stimulated a flow of pancreatic juice of 15 drops in 7½ minutes. The secretin injected above was a stable solution, which had been used in a number of experiments as a control on the animal preparation.

This was compared with the injection of 1 cc. of the secretin used in this experiment in which there resulted a fall of blood pressure from 112 to 50 mm. Hg with a return to the normal height in 6 minutes 20 seconds. This stimulated a flow of 13 drops of pancreatic juice in 7½ minutes.

The injection of 0.0001 gram of histamine intravenously caused a reduction in the blood pressure from 114 to 48 mm. Hg, with a return to normal in 2 minutes 38 seconds. From this injection, 5 drops of pancreatic juice resulted in 7 minutes.

One cubic centimeter of gastrin injected intravenously, caused a fall of blood pressure from 110 to 58 mm. Hg, with a return to normal after 5 minutes. There was no stimulation of pancreatic activity, three drops being formed in 10 minutes, which is but slightly more than normally noted.

When 50 cc. of 0.9 per cent NaCl were introduced into the duodenal loop, there was no fall from normal blood pressure of 110 mm. of mercury, and during the 30-minute period it remained in the loop there was no change in the rate of secretion of pancreatic juice.

The introduction of 50 cc. of secretin into the loop caused no change in the normal blood pressure of 105 mm. Hg. It caused no change in the rate of secretion of the pancreas. The secretin was removed from the loop, the loop washed out with 0.9 per cent NaCl, and the recovered secretin and washings made up to 100 cc.

The injection of 2 cc. of this material intravenously produced a fall in blood pressure from 112 to 48 mm. Hg, with a return to the normal height in 2 minutes 37 seconds. There was only a slight stimulation of pancreatic secretion, four drops being recorded in 6 minutes.

When 50 cc. containing 0.0001 gram histamine were introduced into the intestinal loop, there was a change in the blood pressure from 104 to 95 mm. Hg, but no typical fall. There was no stimulation to pancreatic activity.

The loop containing the histamine was emptied and rinsed out, the rinsings being added to the recovered histamine. Two cubic centimeters were injected intravenously producing a fall in blood pressure from 95 to 38 mm. Hg, with a return to normal in 5 minutes 10 seconds. There was no noticeable effect on the rate of pancreatic secretion following the injection.

Fifty cubic centimeters of gastrin in the duodenal loop caused no change in the blood pressure. There was no secretion of pancreatic juice during the 30 minutes it remained in the duodenal loop.

The gastrin was recovered from the loop and 2 cc. injected intravenously. The blood pressure fell from 108 to 60 mm. Hg with a return to normal in 3 minutes 20 seconds. There was no stimulation to pancreatic secretion.

To show that the loop was capable of absorption and that the animal

was in good condition, 50 cc. of 0.4 per cent HCl were placed into the duodenal loop. There was a very small decrease in blood pressure from 95 to 90 mm. Hg, with a return to normal in 5 minutes, after the mechanical effects had worn off. On the other hand, this 0.4 per cent HCl caused a flow of pancreatic juice of 16 drops in 10 minutes. The stimulation began after the 0.4 per cent HCl had been in the loop 20 minutes.

Following all these experiments, the injection of 1 cc. of the standard secretin previously used caused a fall of 55 mm. Hg, and after a 45 second latent period, there was a flow of 10 drops of pancreatic juice in 7 minutes.

The whole series of experiments carried out as described above was repeated on two other dogs with two different samples of secretin. The results were in every way comparable to the ones just described, and add nothing new to the evidence.

SUMMARY. From the results it is seen that:

1. There is no secretagogue action on the pancreas when secretin, histamine, gastrin or normal salt is in the duodenum. Hydrochloric acid (0.4 per cent) in the duodenum, however, causes the usual marked secretion of the pancreas.

2. There is no change in the blood pressure when secretin, histamine, gastrin, normal salt solution or 0.4 per cent HCl is in the duodenum. Hydrochloric acid acts as a secretagogue without lowering the blood pressure.

3. Both secretagogue and depressor actions are present in the histamine, secretin and gastrin solutions when removed from the duodenum, after having been in the lumen for 30 minutes. The depressor action is possibly very slightly reduced, but the secretagogue action is reduced very decidedly.

DISCUSSION. The fact that the neutral secretin is not absorbed from the intestine confirms the findings of Bayliss and Starling (1), Wertheimer (18) and Matsuo (19) as well as others. That the negative results are not due to the destruction in the intestinal loop is demonstrated by the physiological activity of the recovered material. It was expected that the recovered material would be more active than the original, in view of the fact that aqueous extracts of the duodenum may cause a slight secretion when injected intravenously. The opposite was actually found; there was neither absorption of the neutral material nor extraction of a more active substance from the duodenum. The most obvious conclusion, then, is that there is a physiologically active substance present in the neutral preparation, which if absorbed becomes inactive. Neither is there a substance formed by the neutral extraction of the living duodenum, as shown by the diminished activity of the recovered material, nor which is carried away unaltered by the blood stream, as shown by the negative response of the blood pressure and the secretin while the material was in the loop.

That histamine is not absorbed unaltered is contrary to what was expected in view of the work of Gerard (20) in which he finds the presence of histamine in the loop fluid formed in washed segments of the upper jejunum, the toxic effect of which caused the death of the animal. Symptoms of toxemia developed after 24 hours; on autopsy the intestinal mucosa was found to be necrotic. However, the same worker shows that histamine may be in the closed loop of the colon and cause no toxic symptoms. It appears that factors other than histamine content are involved in the toxemia from closed intestinal loops.

That neither secretin nor histamine is absorbed unaltered from the small intestine indicates that they have some properties in common, but it does not necessarily prove that they are identical. It is probable that neither secretin, as it is usually prepared, nor histamine alone represents the substance (if any such exists) which is responsible for the physiological process of the stimulation of the pancreatic glands during digestion.

GENERAL CONCLUSIONS

1. Secretin solutions prepared from dog duodenal mucosa do not contain histamine in amounts which are of physiological significance.

2. Secretin preparations do not possess their depressor and secretagogue actions as a result of their histamine content.

3. Secretin and histamine are similar, but by no means identical chemically. The latter is much more stable; the former is not precipitable by phosphotungstic acid in active form; also, it is insoluble in amyl alcohol and disappears from the alkaline aqueous solution which was extracted with the amyl alcohol.

4. Secretin and histamine are similar, but by no means identical physiologically. Histamine is the less efficient secretagogue, but the more powerful depressant.

5. Neither secretin nor histamine appear to be absorbed unaltered from the duodenal loop and neither appears to be destroyed therein. This has been shown by physiological assays. Therefore, even if secretin were formed in the natural digestion in the manner claimed by Bayliss and Starling, it would not be absorbed unaltered from a neutral solution.

6. The secretin preparations usually injected do not appear to represent a true physiological process.

7. The losses in secretagogue action as compared with no loss in depressor action observed in the various destructive treatments applied to secretin preparations suggest that the two activities are due to two different substances or different states of the same substance.

The writer wishes to express her indebtedness to Prof. F. C. Koch for valuable aid and counsel throughout the work, and to Dr. M. T. Hanke

for 5 mgm. of pure histamine dihydrochloride. The experimental work on the dogs has been much facilitated by the assistance of Elizabeth Tower.

BIBLIOGRAPHY

(1) Bayliss and Starling: Journ. Physiol., 1902, xxviii, xxix, xxi.
(2) Camus: Journ. physiol. et path. gen., 1902, ix, 998.
(3) Rogers, Rahe, Fawcett, Hackett: This Journal, 1916, xl.
(4) Luckhardt, Henn, Palmer: This Journal, 1922, lix.
(5) Van Eweyk and Tannenbaum: Biochem. Zeitschr., 1921, cxxv.
(6) Schweitzer: Biochem. Zeitschr., 1920, cvii.
(7) Bickel: Berl. klin. Wochenschr., 1917, liv.
(8) Bickel and Eweyk: Sitz. preuss Akad. d. Wissenschaft., 1921, clxxi.
(9) Voegtlin and Meyers: This Journal, 1919–20, xlix; Journ. Pharm. Exper. Therap., 1919, xiii.
(10) Anrep and Drummond: Journ. Physiol., 1920, liv.
(11) Uhlmann: Zeitschr. f. Biol., 1918, lxviii.
(12) Popielski: Pflüger's Arch., 1920, clxxviii.
(13) Abel and Kubota: Journ. Pharm. Exper. Therap., 1919, xiii.
(14) Barger and Dale: Journ. Physiol., 1911, xli.
(15) Koessler and Hanke: Journ. Biol. Chem., 1919, xxxix.
(15b) Koessler and Hanke: Journ. Biol. Chem., 1920, xliii.
(16) Luckhardt and Crogan: Journ. Physiol., 1924, lxviii.
(17) Arthus: Compt. rend. Soc. de Biol., 1918, lxxxi.
(18) Wertheimer and Diwillier: Soc. Biol., 1910, lxviii.
(19) Matsuo: Journ. Physiol., 1912–1913, xlv.
(20) Gerard: Journ. Biol. Chem., 1922, lii.

INSULIN AND BODY TEMPERATURE

MAURICE B. VISSCHER AND ROBERT G. GREEN

From the Departments of Physiology and Bacteriology, University of Minnesota

Received for publication October 17, 1924

The changes in body temperature which occur after insulin injection are of importance to our knowledge of the physiology of insulin. Our interest was aroused by some incidental observations made upon the body temperature of animals after insulin injection, while primarily interested in other reactions. Subsequently, we followed the temperature and blood sugar in a rather large series of experiments.

There are already several reports in the literature upon the effect of insulin upon the body temperature of animals. Our results are recorded because they are somewhat in conflict with those reported previously.

The temperature and blood sugar was followed carefully in thirty experiments upon rabbits and dogs. In each case, between two and five units of insulin per kilogram body weight, a quantity sufficient to cause a marked lowering of the blood sugar, was administered subcutaneously. The blood sugar was estimated by the Folin-Wu method. The blood was not analyzed immediately after drawing each sample, but all the samples from any experiment were analyzed together. This introduced a source of slight error due to glycolysis, but in these results such errors are negligible, because our interest is in the maxima and minima of the sugar curves and not so much in their precise absolute values.

The body temperature observed was always rectal temperature, obtained with calibrated clinical rectal thermometers. Care was taken to insert the stem as well as the bulb of the thermometer in the rectum and each reading recorded represents two concordant readings one minute apart. This precaution eliminated any error from failure to allow the thermometer to arrive at the maximum temperature.

Table 1 contains the important data of our experiments. It has not been possible to tabulate the whole of the temperature curves of each experiment in this way, but the direction and extent of the temperature change has been indicated. Figures 1, 2 and 3 show the complete temperature and blood sugar curves of several representative experiments. Figure 1 shows an instance of a considerable rise in temperature during the first hour after insulin and subsequently a gradual fall. In figure 2 the body temperature is seen to rise gradually over the whole period

of four hours till the rabbit died. In figure 3 there is an example of the slight and gradual fall in temperature that was quite frequently encountered in these experiments.

TABLE 1

EXPERIMENT NUMBER	DATE	ANIMAL	UNITS INSULIN	TIME AFTER ADMINISTRATION FOR MAXIMUM EFFECT	BLOOD SUGAR		TEMPERATURE		REMARKS
					Before insulin	Time of maximum effect	Before insulin	At time of maximum effect	
					per cent	*per cent*	°F.	°F.	
1	6/11/23	Rabbit	5	2 hrs. 20 min.			102.9	98.8	Convulsions
2	6/11/23	Rabbit	5	2 hrs. 30 min.			103.0	98.0	Convulsions
3	6/12/23	Rabbit	5	1 hr. 40 min.			102.5	104.3	Convulsions
4	6/12/23	Rabbit	5	2 hrs.			102.4	100.7	Convulsions
5	6/14/23	Rabbit	5	1 hr. 25 min.	0.17	0.07	102.8	103.45	Convulsions
6	6/14/23	Rabbit	5	1 hr. 15 min.	0.17	0.04	101.8	103.4	Convulsions
7	6/14/23	Rabbit	5	4 hrs. 15 min.	0.22	0.06	102.25	104.8	Convulsions
8	6/14/23	Rabbit	5	1 hr. 15 min.	0.16	0.06	101.7	103.2	Convulsions
9	6/22/23	Rabbit	5	1 hr. 50 min.			103.3	104.5	Convulsions
10	6/22/23	Rabbit	5	1 hr. 50 min.			103.2	104.3	Convulsions
11	8/18/23	Rabbit	4	3 hrs.	0.11	0.025	102.9	104.2	
12	8/28/23	Rabbit	10	2 hrs. 35 min.			103.8	104.55	
13	8/28/23	Rabbit	10	2 hrs. 10 min.			102.4	102.9	
14	8/25/23	Dog, 11 kilos	20	4 hrs. 15 min.			102.3	101.0	Vomiting
15	8/28/23	Dog, 11 kilos	35	2 hrs. 45 min.			102.05	101.05	Vomiting
16	2/28/24	Rabbit	5	4 hrs. 55 min.	0.10	0.04	101.5	99.9	
17	3/ 1/24	Rabbit	5	2 hrs. 45 min.			102.8	96.6	Convulsions
18	3/ 1/24	Rabbit	7	3 hrs.	0.09	0.025	102.5	100.9	Weakness
19	3/ 3/24	Rabbit	5	2 hrs. 15 min.	0.10	0.03	103.9	102.2	
20	3/ 3/24	Rabbit	5	4 hrs.			103.3	101.5	
21	3/ 3/24	Rabbit	5	2 hrs. 10 min.	0.11	0.045	103.3	102.6	
22	3/ 4/24	Rabbit	5	3 hrs.	0.10	0.02	102.8	101.6	Convulsions
23	3/ 4/24	Rabbit	5	2 hrs. 30 min.	0.11	0.02	102.4	100.2	
24	3/20/24	Rabbit	10	1 hr. 25 min.	0.11	0.06	103.9	104.5	
25	3/20/24	Rabbit	10	1 hr. 30 min.	0.10	0.04	103.4	104.1	
26	3/20/24	Rabbit	10	2 hrs.	0.10	0.02	103.8	102.1	Convulsions
27	3/20/24	Rabbit	10	2 hrs. 10 min.	0.10	0.045	103.6	102.4	Convulsions
28	3/21/24	Rabbit	10	3 hrs. 50 min.	0.11	0.02	102.6	100.5	Convulsions
29	3/21/24	Rabbit	10	1 hr. 30 min.	0.08	0.02	104.0	101.3	Convulsions
30	3/26/24	Rabbit	10	1 hr. 40 min.	0.10	0.065	101.6	100.8	

It is evident that there is no uniformity regarding the direction of temperature variation following insulin. The same sample of insulin on the same day caused one rabbit (expt. 4) to show an appreciable fall in body temperature, another (expt. 3) to show an equally great rise in temperature. Convulsions occurred in each of these two experiments.

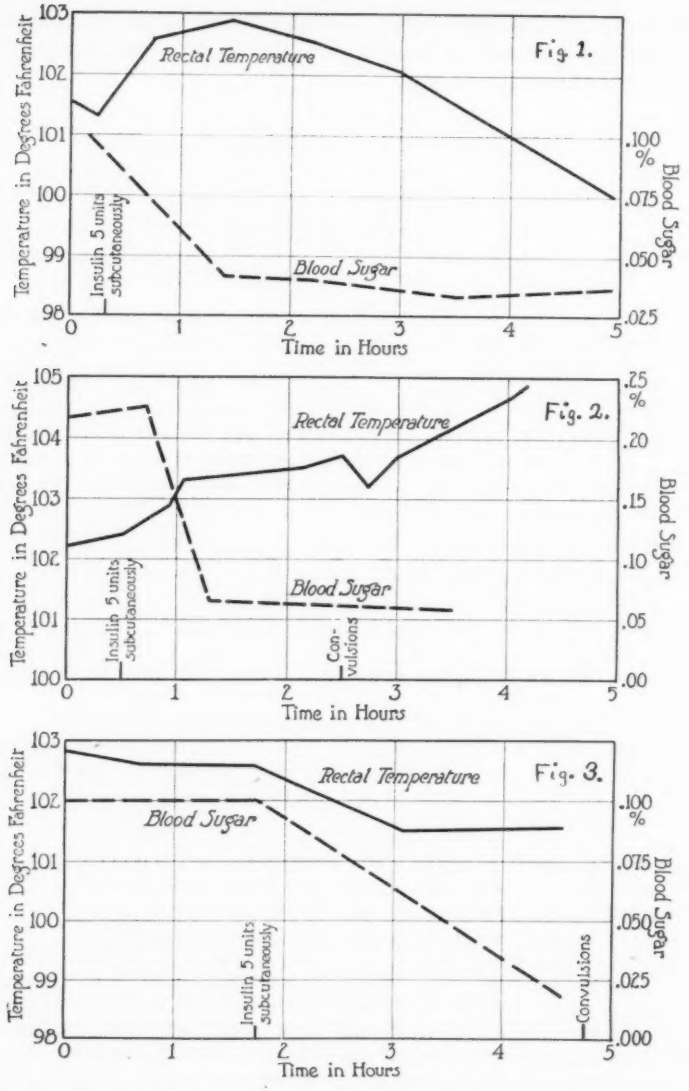


Fig. 1. Experiment 16. A preliminary rise in rectal temperature with subsequent fall is to be observed.

Fig. 2. Experiment 7. A steady rise in rectal temperature is observed. The animal died 4 minutes after last observation.

Fig. 3. Experiment 22. Note only a steady fall in rectal temperature after insulin.

Slight variations of temperature in rabbits are not to be considered as significant since Siebert and Mendel (1) have shown that fluctuations of 0.54°C. (about 1°F.) are physiological. Consequently changes of less than 1°F. have been considered as insignificant in these experiments.

There was as large a proportion of the cases showing a rise in temperature, that showed convulsions, as of the cases showing a fall. Table 2 illustrates this point, as well as showing that the proportion of convulsions in the cases with no temperature change was decidedly smaller.

Dudley, Laidlaw, Trevan and Boock (2) were the first to report a fall in body temperature following insulin. Subsequently Matton (3), Noyons, Bouckaert and Sierens (4) and Arnstein (5) have made similar observations. Our experience would lead us to say that there is no uniformity in the temperature change following insulin. We have observed diametrically opposite fluctuations in rabbits under identical experimental conditions. It appears that the temperature change depends upon some factor other than the pure and simple insulin action. It is possible that the extent of the vascular reaction is the determining factor.

TABLE 2

TOTAL NUMBER OF EXPERIMENTS	EXPERIMENTS WITH TEMPERATURE CHANGE OF LESS THAN ONE DEGREE		EXPERIMENTS WITH FALL OF MORE THAN ONE DEGREE		EXPERIMENTS WITH RISE OF MORE THAN ONE DEGREE	
	Total	Number with convulsions	Total	Number with convulsions	Total	Number with convulsions
30	8	2	15	11	7	6

Edwards and Page (6) have demonstrated the occurrence of marked changes in blood pressure, and have shown a decrease in the ability of the heart to do work, coincident with the hypoglycemic reaction.

The work of Voegtlin and Dunn (7) upon the relation between room temperature and the toxicity of insulin for laboratory animals has shown that the temperature is an important factor in the ability of animals to recover from the insulin reaction. At the present time the relationship is obscure, but it seems probable that it is vascular in its nature.

CONCLUSIONS

The rectal temperature of rabbits has been found to vary, in the majority of instances, after insulin injection. It has not been possible to observe any relation between the blood sugar, or the hypoglycemic reaction, and the temperature. The hypoglycemia may be accompanied by a fall, a rise, or by no change in rectal temperature.

We wish to acknowledge gratefully our indebtedness to the Eli Lilly Company for their generosity in furnishing us with the insulin (Iletin) used in this work.

BIBLIOGRAPHY

(1) SIEBERT AND MENDEL: This Journal, 1923, lxvii, 83.
(2) DUDLEY, LAIDLAW, TREVAN AND BOOCK: Journ. Physiol., 1923, lvii, Proc. xlvii.
(3) MATTON: Compt. rend., 1924, xc, 361.
(4) NOYONS, BOUCKAERT AND SIERENS: Compt. rend., 1924, xc, 365.
(5) ARNSTEIN: Wiener Klin. Wochenschr., 1924, xxxvii, 622.
(6) EDWARDS AND PAGE: This Journal, 1924, lxix, 177.
(7) VOEGTLIN AND DUNN: Pub. Health Repts., 1923, xxxviii, no. 31, 1747.

THE EFFECT OF ADRENALIN ON THE TEMPERATURE OF THE BRAIN

M. W. CASKEY AND W. P. SPENCER

From the Department of Physiology, Ohio State University

Received for publication October 27, 1924

In 1922 Crile and Rowland (1) published a report to the effect that the intravenous injection of adrenalin results in a well-marked increase in the temperature of the brain. The authors interpreted this result as indicating that adrenalin can play a significant rôle in brain metabolism. That the increased temperature reported, however, was actually due to changes in brain metabolism was not convincingly shown. The data as reported did not adequately exclude the possibilities that the change in temperature might have been due either to mass shifting of the blood (2) with consequent perturbations of cerebral circulation or to changes in extra-cerebral tissue metabolism, and especially that of muscle, resulting in elevation of the temperature of the blood as a whole and hence of that reaching the brain. That local circulatory changes in the cerebrum might have been important is indicated by the fact that pressor doses of adrenalin were used. In view of the paucity of cerebral vasoconstrictor fibers the increased systemic pressure presumably resulted in augmented blood flow through the brain. Assuming that heat production in the brain was relatively constant, changes of temperature of the magnitude reported might be brought about merely by increased transportation of the blood from body tissues in which heat production was taking place at a greater rate than in the brain tissue. Even granting the validity of Crile and Rowland's conclusions, however, as referring to rabbits, the investigation is of sufficient importance to demand an extension of the observation to other animals. Accordingly the experiments herewith reported were performed on dogs. The pertinent literature on changes of body temperature and on the use of the thermo-couple in measurement of such changes was reviewed by Crile and Rowland.

METHODS. Dogs of both sexes ranging in weight from 9 to 18 kilograms were used. In each instance carotid blood pressure was recorded throughout the experiment. Ordinarily the distal end of the artery was cannulated, but in a few instances pressures in the central and distal ends were recorded simultaneously. Without further discussion it may be stated that the pressures before, during and succeeding adrenalin injections

were essentially identical in each case. The changes in brain temperature were studied by means of thermocouples. These were made of copper and "advance" wire. The composition of the latter was not precisely known except that it resembles "constan," copper-nickel alloy. The diameter of the advance wire was 0.0188 cm. and of the copper wire 0.024 cm. Pieces about 40 cm. in length were used. The insulation was removed for a short distance from the end of each piece and the ends polished and twisted into a close spiral. The wires of the thermocouple that were to be inserted into the brain were passed through a piece of glass tubing 3 mm. in diameter, drawn to a cone; the juncture projected about 2 mm. The glass was then fused around the juncture and filled with melted paraffin.

This couple was attached in series to a similar couple inserted in a thermos bottle and the terminals attached to a Leeds and Northrup d'Arsonval galvanometer. The apparatus was calibrated as follows. The thermos bottle was filled with water at 37 degrees; the brain couple was placed in a solution in water at 40 degrees and the deflection recorded. The solution was then cooled to 34 degrees and the deflection again recorded. The deflections in the two cases were averaged and divided by three, giving the deflection to be expected for each degree of change. Theoretically this technic is not irreproachable since in the course of a given experiment there was a slight fall in the temperature of the thermos bottle. This change, however, proved to be of relatively such small magnitude and so slow in comparison with the changes of brain temperature as to render it unnecessary to maintain a rigidly constant temperature in the reference couple. Since the fall in the temperature of the thermos bottle was approximately constant in rate a correction formula was easily obtained by dividing the total fall in the thermos bottle temperature by the total length of time of the experiment. This correction has been applied to all the data reported. Galvanometer readings were taken every ten seconds throughout the course of all experiments. At the beginning and at the end of each experiment the galvanometer was short circuited and a zero reading taken. This proved to be constant in each case.

The brain thermocouple was introduced about 2 cm. through a trephine hole into the left hemisphere of the cerebrum. The trephine hole was packed with cotton and the glass tube holding the couple secured firmly in place by threads passed around the head. Adrenalin chloride (Parke, Davis & Co.) was injected by means of a hypodermic syringe into the basilary vein. The animals were anesthetized with ether in all cases by trachea, using an ordinary ether bottle.

DATA. A preliminary orienting study was made of the simultaneous effects of adrenalin upon blood pressure and brain temperature. In this study sixty-one experiments were made upon seven dogs. It became apparent at once that an indubitable rise in cerebral temperature occurs

and that this rise does not synchronize with augmented carotid pressure. More specifically it was noted that during the first minute after the injection of adrenalin the brain temperature fell rapidly, generally reaching the lowest point in one minute and thirty seconds or at most in two minutes after the injection, where dosages of 0.015 mgm. per kilo or more were used. With dosages smaller than this the drop in temperature was sometimes noted, but it was correspondingly less in magnitude and other factors which tended to affect the temperature slightly, such as sensory stimulation, made it difficult to determine whether this effect was constant with small dosages.

This fall in temperature seldom exceeded one tenth of a degree Centigrade. There was no constant relationship between the fall in temperature and carotid pressure changes except that they began almost simultaneously. In figure 1, for example, is shown a case in which the brain temperature fell during the first pressor phase and the "dip" and then started upward while blood pressure was on an upward slanting plateau to pass the initial temperature while blood pressure was still rising. The temperature continued to rise during the secondary depressor phase. The blood pressure and brain temperature then began almost simultaneously to return to their initial lines, which were reached at the end of twelve minutes. The brain temperature continued to fall for five minutes and continued below the initial temperature for six minutes longer when the observation was discontinued. In figure 2 is shown an instance in which the fall in brain temperature and the rise in blood pressure gave almost simultaneous reciprocal curves through a period of two minutes; but the phase of augmented cerebral temperature persisted through the following three minutes while blood pressure remained at its initial level. The greatest fall in brain temperature noted was 0.252°C. at a period 2.5 minutes after the adrenalin injection. In this case the highest temperature reached was 0.007°C. above the initial level, at a period 7 minutes and 10 seconds after the injection. The greatest rise of brain temperature noted was 0.336°C. at 7 minutes and 50 seconds after the adrenalin injection and while the blood pressure was below the initial level. The maximum temperature was sometimes maintained for a period of several minutes but generally began to fall within a minute and continued falling steadily until it reached a point considerably below that of the initial temperature. It has been pointed out by Crile (3) that the brain temperature falls steadily under ether anesthesia and our findings corroborate this point. It would seem then that in determining the effect of adrenalin on brain temperature this factor should be taken into consideration. The initial fall due to adrenalin would be very slightly less than the recorded data show and the rise considerably higher. But in view of the difficulty of determining the exact extent to which this factor was present during the

time in which brain temperature was fluctuating no such corrections were made for the data.

A comparison of blood pressure and brain temperature graphs shows that the maximum brain temperature is generally reached soon after the blood pressure has fallen to its lowest point. With large doses the blood pressure is maintained at a high level for a considerable period of time and during this time brain temperature does not rise. While the blood pressure is beginning to fall the brain temperature is beginning to rise. When doses of 0.0033 mgm. per kilo and less were used the effect on brain temperature was so slight, if present at all, that it could not be accurately detected, especially as other factors caused slight fluctuations. As

TABLE 1

Fluctuations in brain temperature and blood pressure after the injection of adrenalin chloride

NO.	DOSAGE PER KILO OF ADRENALIN CHLORIDE, 1 : 1000	FALL IN BRAIN TEMPERATURE BELOW INITIAL TEMPERATURE	TIME INTERVAL BETWEEN INJECTION OF ADRENALIN AND MINIMAL BRAIN TEMPERATURE	RISE IN BRAIN TEMPERATURE ABOVE INITIAL TEMPERATURE	TIME INTERVAL BETWEEN INJECTION OF ADRENALIN AND MAXIMAL BRAIN TEMPERATURE	RISE IN BLOOD PRESSURE IN MILLIMETERS OF MERCURY ABOVE INITIAL PRESSURE	TIME INTERVAL BETWEEN INJECTION OF ADRENALIN AND HIGHEST BLOOD PRESSURE	FALL IN BLOOD PRESSURE IN MILLIMETERS OF MERCURY BELOW INITIAL PRESSURE	TIME INTERVAL BETWEEN INJECTION OF ADRENALIN AND LOWEST BLOOD PRESSURE
	mgm.	*°C.*		*°C.*		*mm.* Hg		*mm.* Hg	
1	0.15	0.056	1′50″	0.175	9′10″	54	30″	24	5′30″
2	0.15	0.049	1′20″	0.154	9′20″	56	2′	38	8′20″
3	0.075	0.077	1′20″	0.112	7′	126	20″	30	4′40″
4	0.075	0.105	1′20″	0.133	6′20″	80	20″	42	6′
5	0.03	0.035	1′40″	0.154	4′50″	76	50″	46	4′30″
6	0.03	0.042	1′10″	0.147	5′10″	64	1′	40	3′30″
7	0.015	0.007	50″	0.189	4′	68	30″	32	3′10″
8	0.015	0.007	50″	0.147	3′50″	72	50″	24	2′50″
9	0.0075			0.042	3′	46	1′		
10	0.0075			0.105	2′40″	46	1′10″	8	3′

increasingly smaller doses were used, not only were the blood pressure and brain temperature correspondingly less affected, but the changes took place more rapidly. Thus while the effect of a 0.15 mgm. injection could sometimes be noted for a half-hour, a 0.0033 mgm. dose produced its total effect in two or three minutes.

Table 1 gives data from simultaneous blood pressure and brain temperature records which were chosen as typical for varying dosages of adrenalin from this series of preliminary experiments. Figures 1, 2 and 4 are graphs of brain temperature and blood pressure superimposed in order to facilitate comparison.

The experiments reported above establish the time relationship between

the changes in carotid blood pressure and in brain temperature after the injection of varying dosages of adrenalin. They show that the marked rise in brain temperature cannot be due to an increased flow of blood through the brain during the time of rise, since the brain temperature falls during the time when blood pressure is highest in the carotid and rises to its maximum during the secondary depressor period.

It might seem likely that the same vasoconstrictor effect that was raising carotid pressure was at work in the vessels of the brain as well as in other organs and that for this reason we might expect to find less blood flow in the brain during the time of high blood pressure. Wiggers (4) in 1905 and again (5) in 1914 gives the results of experiments on perfusing the brain with a pulsating Locke's solution and injecting adrenalin, and shows that there is a vasoconstriction in the brain. But he admits that this vasoconstrictor effect is probably not marked enough to counteract the effect of the heightened pressure in the arteries supplying the brain and that the result is an increased flow in the brain during the pressor phase. Dixon and Halliburton (6) present proof that the cerebral vessels possess no efficient vasomotor nerves and the researches of Biedl and Reiner, Hill, Macleod and others confirm this view.

In substantiation of the above work, and in order to make sure that there was no obstruction in the circulation through the Circle of Willis after operating, we took the blood pressure simultaneously in the peripheral and central ends of the left carotid artery. The four graphs which we secured, using dosages of 0.0166 mgm. and 0.033 mgm. per kilo, all showed an equal and simultaneous rise in blood pressure in both ends of the carotid. This revealed a free circulation through the Circle of Willis and no appreciable vasoconstriction in the arteries anastomosing to form the Circle.

The time relationships established between pressure changes and temperature changes in the brain made it clear that the fluctuations in brain temperature after the injection of adrenalin chloride, and in particular the marked rise in brain temperature, could not be explained on the ground of varying flow of blood in the brain. In order to make certain that the changes in brain temperature were not secondary effects due to the circulation it remained to determine whether any fluctuations occur in the temperature of the blood passing to the brain, to establish the time and quantity relationships between these fluctuations, if present, and the changes in brain temperature. It was likewise necessary to estimate the value of such a comparison between carotid and brain temperature in order to interpret the temperature changes going on in the brain.

Preliminary to the work on carotid temperature we took four records of the temperature of the muscles after injection of adrenalin. We inserted a thermocouple under the sartorius. The results of Boothby and Sandiford (7) in which it is shown that body metabolism increases 20 per

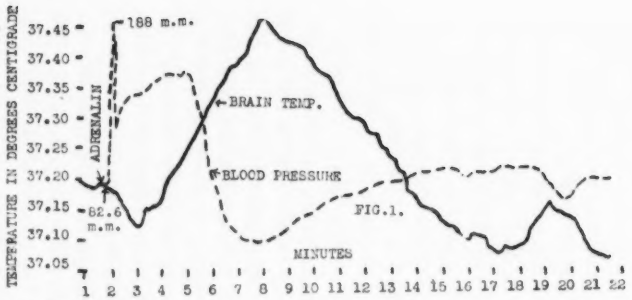


FIG. 1.

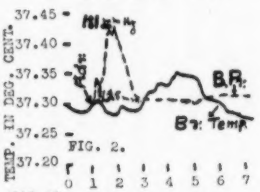


FIG. 2.

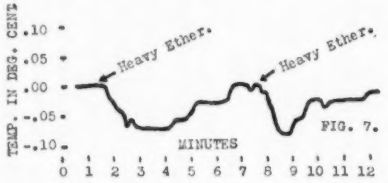


FIG. 7.

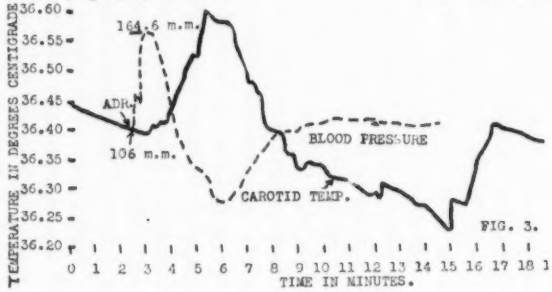


FIG. 3.

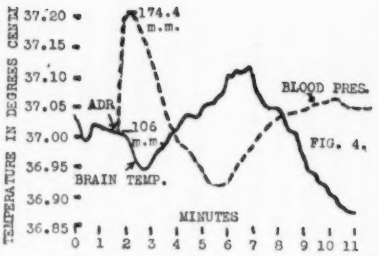


FIG. 4.

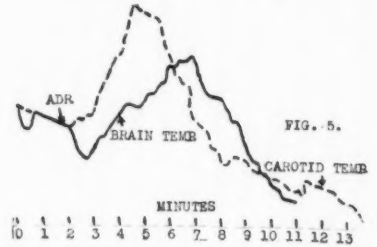


FIG. 5.

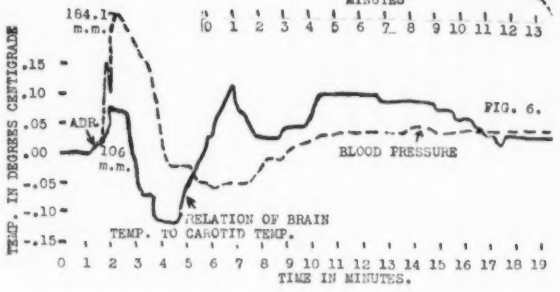


FIG. 6.

cent as a result of the subcutaneous injection of 0.5 mgm. of adrenalin chloride, led us to believe that some of this heat was being produced in the muscles. Contrary to the results of Crile, we secured a distinct rise in muscle temperature after adrenalin chloride had been injected.

In these experiments on carotid temperature six dogs, four male and two female, were used. The same operative technique and anesthesia were used as in previous work on brain temperature. Blood pressure from the left carotid was recorded in each case. One thermocouple was introduced into the brain and a second one, not enclosed in glass, was carefully bent around the right carotid, and held in place by cotton packing and rubber tubing. Extreme care was taken to secure a direct contact between the thermal junction and the wall of the carotid and in every case the packing and position of the thermal junction were inspected after the experiment. These two couples were connected together and also connected to a third couple which was immersed in the thermos solution at body temperature. With this set-up any two of the thermal junctions could be thrown simultaneously in circuit with the galvanometer. Either the brain temperature changes, the carotid blood temperature changes, or the relative fluctuations of brain and carotid blood temperatures could be recorded.

Table 2 shows the results of eleven injections of adrenalin chloride on the temperature of the blood in the carotid. Figure 3 is reconstructed directly from the galvanometer readings of carotid temperature which were recorded at ten-second intervals and from the blood pressure record taken simultaneously on a kymograph. A study of the data recorded in table 2 shows that in every case in which carotid temperature was taken after the injection of adrenalin there was a distinct rise. In a number of cases this rise was small and by a comparison of these data with those in table 1 it may be seen that in general the rise in brain temperature is higher than that in carotid temperature when the same dosage of adrenalin is used. But this is not invariably the case and it cannot be said that the blood temperature never goes higher than does the brain temperature after injection of adrenalin chloride. On comparison of these tables another point seems significant. For the same dosage of adrenalin chloride the maximum blood temperature is almost if not always reached considerably

Fig. 1. Male dog; body weight, 10.2 kgm.; adrenalin dosage, 0.075 mgm. per kilo.

Fig. 2. Male dog; body weight, 16.5 kgm.; adrenalin dosage, 0.0075 mgm. per kilo.

Fig. 3. Female dog; body weight, 9.8 kgm.; adrenalin dosage, 0.05 mgm. per kilo.

Fig. 4. Female dog; body weight, 9.8 kgm.; adrenalin dosage, 0.05 mgm. per kilo.

Fig. 5. Combination of carotid blood temperature graph from figure 3 and brain temperature graph from figure 4.

Fig. 6. Female dog; body weight, 9.8 kgm.; adrenalin dosage, 0.05 mgm. per kilo.

Fig. 7. Male dog; body weight, 13.6 kgm.; graph of relation of brain temperature to carotid temperature, showing relative fall in brain temperature during deep anesthesia.

sooner than the maximum brain temperature. In comparing these physiological time relations it seems better to use the blood pressure record as a time measurement than to count time in minutes and seconds. In all experiments we took blood pressure records and on referring the graphs on brain temperature to their corresponding blood pressure graphs and the graphs on blood temperature to their blood pressure graphs we find that the maximum blood temperature comes at a distinctly earlier point on the blood pressure record than does the maximum brain temperature.

A series of twelve records was taken of the galvanometer readings with one junction in the brain and the other on the carotid. The results of these experiments are summarized in table 3. A word of explanation is necessary in order to make clear what this table represents. The relation

TABLE 2

Rise in the temperature of the carotid artery after injection of adrenalin

NUMBER	DOSAGE PER KILO OF ADRENALIN, 1 : 1000	RISE IN CAROTID TEMPERATURE	TIME INTERVAL BETWEEN INJECTION OF ADRENALIN AND MAXIMUM CAROTID TEMPERATURE	RISE IN BLOOD PRESSURE IN MILLIMETERS OF MERCURY ABOVE INITIAL PRESSURE	TIME INTERVAL BETWEEN INJECTION OF ADRENALIN AND HIGHEST BLOOD PRESSURE
	cc.	°*C.*		*mm.* Hg	
1	0.03	0.140	1′ 40″	68	15″
2	0.04	0.042	1′ 50″	44	10″
3	0.04	0.084	4′ 40″	48	10″
4	0.04	0.084	1′ 40″	44	30″
5	0.04	0.063	2′ 20″	56	50″
6	0.045	0.049	2′ 20″	62	10″
7	0.046	0.091	1′ 50″	84	10″
8	0.046	0.203	1′ 30″	70	20″
9	0.046	0.189	2′ 30″	62	25″
10	0.05	0.196	2′ 40″	56	30″
11	0.073	0.189	3′ 40″	100	50″

of brain to carotid temperature is shown at one minute intervals for ten minutes following the injection of adrenalin. The relation of brain temperature to carotid temperature at the time of the injection is taken as zero and any fluctuation in this relation from minute to minute is given in degrees centigrade. The graphs from which this table is made show the fluctuations of brain and carotid temperature in relation to each other and not the actual rise or fall in either. When the brain temperature is higher than the carotid temperature, in comparison to their initial relation, this is shown by plus figures. When the carotid temperature in comparison to the brain temperature is relatively higher than the initial relation the figures are minus. The temperature might be falling in both brain and carotid throughout the experiment and if it were falling more rapidly in the carotid this would be shown by increasingly larger plus figures in the table,

or by an ascending line on the graph. Figures 3 and 4 show respectively carotid and brain temperature changes after the injection of the same dosage of adrenalin chloride. Figure 5 shows these two superimposed and if a reconstruction graph showing the change in relation between brain and carotid temperature in the graphs in figure 5 were made it would appear very much like the graph in figure 6, which is made up from the readings secured when one thermocouple was in the brain and the other on the carotid.

A study of table 3 makes it clear that the actual results, when the relationships of the fluctuations of carotid and brain temperature are plotted from the galvanometer readings with brain and carotid couples in the circuit, are about what we would have expected from our comparison of the tables of brain temperature and of carotid temperature taken independently. In most cases the temperature of the brain goes up in comparison to that of the carotid. There is, however, in eight of the twelve records a period of relative drop in brain temperature in comparison to that of the carotid. This corresponds to the time when we would expect, from our other data, that carotid temperature was at or near its maximum, while brain temperature was only starting to rise. In one case, no. 12, the carotid temperature was at all times higher in relation to brain temperature than the initial relation.

A fact which might explain the results in record 12 is the very marked effect of deep ether anesthesia on brain temperature in comparison to any slight effect (if indeed there be such) on blood temperature. It was repeatedly noted during the time when brain and carotid temperature relationships were being recorded that heavy anesthesia immediately resulted in a marked fall of brain temperature in relation to blood temperature. In several instances deep ether was marked in our record book and figure 7 illustrates this typical effect. This is what we might expect from the fact that the nerve cell contains so much ether soluble lipoid in comparison to other cells. It is possible that the animal was under unusually deep ether anesthesia when record 12 was made.

The data in table 3 lead to the conclusion that the effect of adrenalin chloride on brain temperature is in part at least a primary effect, resulting from an increased rate of tissue metabolism in the brain and the production of a very considerable amount of heat. It might be thought possible that all of the fluctuations in temperature relations between the carotid blood and the brain could be explained as due to slow circulation through the brain. The blood at maximum temperature might penetrate the brain tissue at a slow rate and thus when cooler blood was in the carotid the warm blood would be in the brain. Such an explanation hardly seems to fit the facts. It would not account for certain records in which the relationship of brain temperature to carotid temperature was at all times higher than

TABLE 3

Relationship of brain temperature to carotid temperature in degrees Centigrade

Plus figures denote relative rise in brain temperature in comparison to carotid temperature. Minus figures denote relative fall in brain temperature in comparison to carotid temperature. One minute intervals for 10 minutes after the injection of adrenalin.

NUMBER	DOSAGE PER KILO OF 1:1000	1 MINUTE	2 MINUTES	3 MINUTES	4 MINUTES	5 MINUTES	6 MINUTES	7 MINUTES	8 MINUTES	9 MINUTES	10 MINUTES
	cc.										
1	0.023	+0.028	+0.014	+0.042	+0.056	+0.070	+0.028	−0.028			
2	0.04	+0.028	+0.071	+0.163	+0.255	+0.269	+0.248	+0.220	+0.178	+0.164	+0.178
3	0.04	+0.056	+0.098	+0.126	+0.056	+0.028	0.000	0.000	−0.028	−0.042	−0.056
4	0.04	+0.042	0.000	+0.070	+0.014	−0.014	−0.084	−0.224	−0.168	−0.140	−0.098
5	0.04	+0.084	+0.112	+0.119	+0.168	+0.147	+0.091	+0.021	+0.049	+0.105	+0.105
6	0.04	0.000	+0.014	+0.084	+0.112	+0.098	+0.056	+0.042	+0.056	0.000	0.000
7	0.05	+0.014	0.000	+0.028	+0.084	+0.084	+0.112	+0.140	+0.168	+0.168	+0.140
8	0.05	+0.035	−0.056	−0.021	+0.049	+0.126	+0.182	+0.182	+0.182	+0.154	+0.168
9	0.05	0.000	−0.084	−0.126	−0.028	+0.077	+0.014	+0.014	+0.028	+0.077	+0.077
10	0.073	−0.070	−0.098	−0.042	−0.028	+0.014	0.000	+0.035	−0.042	−0.028	−0.028
11	0.073	−0.056	−0.070	−0.042	−0.028	0.000	+0.014	−0.014	−0.014	−0.042	−0.028
12	0.046	0.000	−0.063	−0.112	−0.154	−0.210	−0.182	−0.182	−0.154	−0.154	−0.140

the initial relation at the time when adrenalin chloride was injected. The sudden fluctuations of brain temperature due to ether anesthesia cannot be accounted for by fluctuations in blood temperature. The work of Hill (8) on heat production in the nerve fibers seems to show that there is little or no rise in temperature in the fibers during the passage of an impulse. This, however, does not preclude the possibility of the nerve cell bodies carrying on oxidative processes more or less comparable to those of muscle cells in quantity of heat produced.

SUMMARY

1. The intravenous injection of adrenalin chloride in dogs is followed by a sudden, comparatively small, fall and subsequent relatively large rise in brain temperature.

2. This effect seems to bear a constant time relation to the effect of adrenalin on blood pressure, the drop in brain temperature occurring during the period of high blood pressure and the rise occurring during the secondary depressor effect on blood pressure or soon after it.

3. In general the amplitude of the temperature changes in the brain and of the changes in blood pressure are proportional to the size of the dosage used in amounts ranging from 0.2 mgm. to 0.00015 mgm. per kilo body weight.

4. The smaller the dosage the quicker is its effect on brain temperature and blood pressure.

5. The injection of adrenalin chloride is followed by a marked rise in muscle temperature.

6. The temperature of the blood in the carotid artery rises after the injection of adrenalin chloride. This rise in temperature occurs sooner than the rise in brain temperature and in most cases is not as great.

7. Ether anesthesia affects the brain temperature much more than it does the blood temperature.

8. The temperature changes in the brain after the injection of adrenalin chloride cannot be due entirely to changes in blood temperature and volume, and are produced in part at least by changes in the rate of oxidation in the brain tissue.

The authors wish here to express their appreciation to Dr. R. G. Hoskins for his interest in, and direction of, this work.

BIBLIOGRAPHY

(1) Crile, Hosmer and Rowland: This Journal, 1922, lxii, 341.

(2) Hartman and McPhedran: This Journal, 1915, xxxviii, 438. Hartman and McPhedran: Ibid., 1917, xliii, 311. Hoskins, Gunning and Berry: Ibid., 1916, xli, 513.

(3) CRILE AND ROWLAND: This Journal, 1922, lxii, 349.
(4) WIGGERS: This Journal, 1905, xiv, 452.
(5) WIGGERS: Journ. Physiol., 1914, xlviii, 109.
(6) DIXON AND HALLIBURTON: Journ. Physiol., 1914, xlviii, 128.
(7) BOOTHBY AND SANDIFORD: This Journal, 1923, lxvi, 93.
(8) HILL: Journ. Physiol., 1912, xliii, 433.

STUDIES IN PULSE WAVE VELOCITY

III. Pulse Wave Velocity in Pathological Conditions

JANE SANDS[1]

From the Department of Physiology, University of Pennsylvania, the Philadelphia General Hospital and the Department of Therapeutics, University of Edinburgh

Received for publication October 29, 1924

In connection with the investigations of this subject already reported (1), (2), it seemed advisable to investigate the pulse wave velocity in pathological conditions.

All the records were taken using the technique described previously (1). Certain difficulties were encountered: some of the cases were senile, in the majority of patients with arterio-sclerosis the apex beat could not be palpated and often the heart sounds were so feeble that they were inaudible with a stethoscope. On the other hand the apex thrust and the sounds in patients with aortic disease and hyperthyroidism were so violent that the records were apt to be confused unless read with great care.

In order to increase our knowledge of the normal pulse wave velocity, a further series of eight normal individuals was examined. Table 1 gives in detail the results obtained and in table 2 are grouped the results of all the normal cases I have investigated. From this data I find that the average normal velocity from the heart to the radial artery is 4.90 meters per second, while that from the heart to the dorsalis pedis is 5.61 meters per second. It has been found with changes of arterial elasticity, consequent on increasing age, that there is a tendency for the velocity to increase (3), (4). Consequently the pulse wave velocity will depend upon the age of the individual studied.

A comparison of these figures with those obtained from Bramwell, Hill and McSwiney (3) and by Bramwell (4) shows that my figures are always lower (age being considered). However, in both series there is a proportionate increase in the velocity with advancing years. These figures are not strictly comparable since the above authors have based their results upon a comparison between the carotid and the radial, which excludes any effect from the aorta. Bazett and Dreyer (5) have shown that the velocity is less in the larger central vessels and greater in the peripheral vessels. Therefore records including the aorta, as those measuring from apex beat

[1] Medical Fellow of the National Research Council, U. S. A.

TABLE 1

Complete data from a series of eight normal individuals

CASE NUMBER	SEX	AGE	BLOOD PRESSURE		P. P.	P. R.	APEX TO BRACHIAL		BRACHIAL TO RADIAL		APEX TO RADIAL		APEX TO FEMORAL	FEMORAL TO DORSALIS PEDIS	APEX TO DORSALIS PEDIS	AVERAGE A-R
			Systolic	Diastolic			Right	Left	Right	Left	Right	Left				
1	M.	16	132	79	53	60	3.32	3.13	4.77	5.69	3.62	3.58	3.57	8.55	5.00	3.60
2	M.	17	118	46	72	72	3.43	3.66	5.64	4.74	3.94	3.90	3.38	8.30	5.41	3.92
3	M.	19	131	56	75	60	2.61	2.52	4.10	4.90	2.93	3.02	2.75	9.25	4.90	2.98
4	M.	23	110	80	30	72	3.28	3.29	7.30	7.04	4.04	4.01	3.69	11.44	6.36	4.03
5	F.	26	116	78	38	96	5.56	5.00	4.90	4.90	5.31	4.95			5.80	5.13
6	F.	25	110	55	55	84	4.40	3.96	4.66	3.60	4.48	3.62			5.85	4.05
7	F.	27	115	78	37	72	2.90	3.26	5.90	9.16	3.64	3.98			4.75	3.81
8	M.	41	164	108	56	72	5.19	5.28	13.21	12.76	6.85	7.06			6.45	6.95
Average		24.25	126.5	71.4	55.1	73.5	3.836	3.762	6.31	6.598	4.35	4.265	3.35	9.385	5.57	4.31

TABLE 2

Apex to brachial, radial, femoral and dorsalis pedis in normal individuals

A-Br.	Br.-R	A-R	A-F	F-DP	A-DP	NUMBER OF CASES	AGE LIMIT	AVERAGE AGE	REMARKS	REFERENCE
4.6	8.8	5.5				7	19–25	22		Sands—from article by Bazett and Dreyer—THIS JOURNAL, 1922–23, lxiii, 102
3.9						22	20–30	25		Sands—THIS JOURNAL, 1923, lxvii, 210
3.12						1		29	37 cycles	Sands—THIS JOURNAL, 1923, lxvii, 206
			4.5	7.5	5.65	13	18–26	23		Matzke, Priestley and Sands—THIS JOURNAL, 1923, lxvii, 217
3.87	8.8	5.5	4.5	7.5	5.65	43	18–30	25	Averages of above cases	
3.80	6.5	4.31	3.35	9.38	5.57	8	16–41	24–25	Averages of table 1	
3.84	7.66	**4.90**	3.93	8.44	**5.61**	51	16–41	25	General averages of all cases	

or the R wave of the electrocardiograph to the peripheral vessels, would have a slower velocity than those dealing only with two peripheral vessels.

Records from cases of arterio-sclerosis. The outstanding observations in the condition are a variation from the normal both in large and small vessels, while the variation in central and peripheral vessels is not necessarily of the same pathological character. The changes in the pulse wave velocities of central and peripheral vessels in the upper and lower extremities are similar; also the relatively more rapid rate in the lower extremity as compared with the upper is maintained as in the normal.

For reasons already mentioned, apex beat curves were difficult or impossible to read in some of these cases, therefore some of the figures given are those calculated from the R wave of the electrocardiogram with an allowance of 0.05 second for rising tension time. This has been found to be the average time interval in similar subjects where the apex curves are readable (1). There is also a correction of 0.01 second where the electrical and mechanical curves are compared.

It was apparent that at least two types of cases were to be found. In a number of instances there was a slowing of the velocity in the peripheral vessels, which was sometimes associated with an increased rate in the central vessels, the combined velocity of the two being as slow or slower than the average normal figure, even if the age factor was neglected. In other cases the pulse wave velocity was increased in both central and peripheral vessels. Because of the presence of these types the results have been separated into two groups. They are incorporated in tables 3 and 4 according to whether the velocity was increased above normal or decreased.

In attempting to explain these diverse results in the pulse wave velocity many factors must be taken into consideration. The expansibility of the arterial walls is one of the chief factors, but forces such as the effective pressure in the vessel, the size and irregularities of the lumen and the tortuosity of the vessel all seem to enter into the problem.

In order to determine the effect of these factors a circulation scheme was arranged, full results of which are about to be published (Jonathan Meakins, D. Murray Lyon and Jane Sands). An air pump (described by Dickson (6)) driven by an electric motor was connected through a mercury manometer with a series of valves and a system of rubber tubing which was filled with a fluid, with a reservoir in the circuit. By such a mechanism the fluid was made to circulate by intermittent pulsation and the volume of fluid was constant, since the fluid forced out past the upper valve returned by way of the reservoir through the lower valve opening. Optical records of the wave velocity were taken using exactly the same apparatus as was used for examining patients.

The first experiment was designed to study the effect of changes in the

TABLE 3

Pulse wave velocity in arterio-sclerosis

CASE NUMBER	AGE	BLOOD PRESSURE		P. R.	ARM			LEG			REMARKS
		Systolic	Diastolic		A-Br.	Br.-A	A-R	A-F	F-DP	A-DP	
13	55	170	100	72	5.64*	5.49	5.53				Vessels thickened
14	59	100	70	84	6.83	6.32	6.55	10.77		8.81	General sclerosis; marked beading
15	68	105	80	75	4.09	3.27	3.70	5.24	16.89	8.42	Vessels tortuous and beaded
16	70	120	90	90	4.84*	7.15	5.46				Vessels thick
17	70	110	92	30	*		7.45	13.58	17.10	15.20	Vessels very stiff; heart block
18	65	110	90	96	9.10*	4.91	6.24				Vessels thick
19	38	210	130	96	5.17	11.66	6.31	5.50	21.6	9.30	Vessels slightly thickened
20	76	150	120	132	4.45	7.95	5.34				Vessels thick, not tortuous; pulsus alternans
21 14/2/24	45	185	135	78	4.04	8.80	5.19	5.37	15.08	8.67	Vessels thick—muscular
21 19/2/24		170	130	72	4.53	8.77	5.34	5.61	15.90	9.36	
21 26/3/24		182	140	88	4.89	10.68	5.94	4.92	12.42	8.24	
22	57	190	115	96			4.44			9.61	Vessels stiff and tortuous; cardiac hypertrophy
23	60	135	80	76	6.62	10.48	6.32	7.80	10.25	8.88	Vessels slightly stiff
24	74	102	60	60	4.37	9.90	5.61	4.95	8.94	6.75	Vessels stiff and tortuous
25	49	128	90	72	3.62	6.60	5.65	6.17	6.54	6.38	Vessels thick, not stiff, not tortuous
26	70	138	74		6.79	4.36	5.10	10.63	7.66	8.75	Vessels stiff, very thick and beaded. One leg amputated for gangrene

* Denotes that the calculation is from the R wave rather than the apex beat.

TABLE 4

Pulse wave velocity in arterio-sclerosis

CASE NUMBER	AGE	BLOOD PRESSURE		P. R.	ARM			LEG			REMARKS
		Systolic	Diastolic		A-Br.	Br.-R	A-R	A-F	F-DP	A-DP	
1	60	200	128	72	7.03*	4.42	5.76	7.02	5.50	6.05	Vessels thick and tortuous
2	76	220	130	50	5.12	4.28	4.49	6.24	19.21	10.13	Vessels tortuous, beaded; mitral stenosis
3	60	176	100	72	4.39	3.48	3.96	5.65	10.90	7.77	Vessels tortuous, beaded
4	54	180	110	72	5.83	3.51	4.68			6.28	Vessels tortuous, thickened
5	53	154	110	80	7.82*	2.85	4.71				Vessels thick, not tortuous
6	50	184	100	80	3.94*	1.12	2.30	4.67	5.55	5.36	Vessels stiff, not tortuous
7	71	140	100	60	2.88	2.25	2.69	3.80	8.52	5.55	Vessels very tortuous; mitral stenosis
8	53	110	80	60	4.95	3.10	3.86	4.34	6.66	5.50	Vessels stiff, tortuous, beaded
9	57	118	78	75	4.42	5.26	4.67	5.45	5.40	5.76	Vessels stiff, large tortuous waves
10	43	144	100	120	4.53	1.95	4.85	6.45	6.49	6.48	Vessels thick, large tortuous. Angina
11	40	115	80	84	5.25	3.60	4.72	4.68	7.29	5.40	Vessels stiff and tortuous
12 (17/3/24)	45	192	120	90	3.62	7.28	4.25	4.15	8.17	5.87	Vessels somewhat stiff; cardiac hypertrophy
12 (18/3/24)		185	125	70	3.46	8.88	4.10				
12 (20/3/24)		190	135	84	3.64	8.97	4.76			6.57	

* Denotes that the calculation is from the R wave rather than the apex beat.

diameter of the tubes, the walls being constant. New rubber tubes of the same make and quality, each having a wall 1 mm. in thickness but having varying bores, were used. The following tabulation shows the results:

TUBE	DIAMETER	PULSE WAVE VELOCITY PER SECOND
	mm.	*meters*
1	3	16.26
2	4	13.78
3	5	12.54

From these data we find that the wave velocity is faster in smaller tubes (the walls remaining constant). On the other hand in tubes where the diameter of the bore was constant and the thickness of the walls varied, the velocity was faster with the thicker wall changing from 28.86 meters per second in a 1.5 mm. wall to 28.05 meters per second in a 3 mm. wall. These observations are in exact agreement with the formula developed by Moens (8).

Another series of experiments was designed to show the effect of change of wave rate on the velocity. No difference in the rate of conduction could be demonstrated. For example, in a typical experiment the wave velocity at a wave rate of 92 per minute was 13.38 meters per second while at 150 per minute it was 13.52 meters per second.

To test the effect of tortuosity the tube was threaded through the posts of a test tube rack in such a manner as to avoid stretching. The recording tambours were placed on the tube at the same points used when the tube was straight. Findings from three such experiments on different tubes are given in table 5.

TABLE 5

Schema: To show the effect of tortuosity

Tube 3

STRAIGHT	FEW LARGE TURNS	MANY SMALLER TURNS CAUSING IRREGULARITIES OF THE LUMEN
13.13	11.91	
12.24	11.85	9.28
13.90	12.5	11.1

These figures clearly show that tortuosity of the tube retards the actual velocity of the wave.

The experiments of Bramwell and Hill led them to conclude that with an increase of pressure within an excised artery the wave velocity was increased. Our experiments would indicate that this is not necessarily the dominant factor. These results will be the subject of a future communication.

In a recent paper Bramwell (4) makes the following statements:

> The variations observed in pulse-wave velocity show that the elasticity of the arteries is affected to a very marked degree by different physiological and pathological conditions. The more perfect the elasticity of the arteries the greater is their change of volume for a given rise or fall of pressure. Hence from the point of view both of the heart and the capillary circulation arterial elasticity is a factor of fundamental importance to the circulatory mechanism.
>
> The influence of blood pressure on arterial elasticity is discussed. . . . . It was found that at low pressures healthy arteries are extremely elastic but that as the pressure rises above the normal diastolic value their elasticity rapidly diminishes. These facts point to the conclusion that in healthy young subjects during life the elasticity of the arteries is largely dependent on the diastolic pressure. The higher the diastolic pressure, the less efficient will be the arteries and the greater will be the energy required of the heart in ejecting its contents.
>
> In disease, on the other hand, not only is the elasticity of the vessel wall impaired but the characteristic relationship of elasticity to pressure which exists in health is no longer present.

The statement that in disease the relation of elasticity to pressure, which exists in health, is lost, seems to be evident from the data of cases of arterio-sclerosis. In relation to pulse wave velocity, however, several factors are probably active and the loss of expansibility may be overbalanced by other forces.

The larger central vessels become less expansible but do not readily become tortuous, the diameter is not materially changed and therefore the velocity tends to increase. The smaller vessels also become less expansible and where this is the primary factor the peripheral velocity is markedly increased. The slowing of the pulse wave velocity in other cases might be due to effects produced by irregularities of the lumen and tortuosity great enough to overbalance the effect of loss of expansibility of the walls. In addition the slow rate of transmission may be sometimes apparent only, since it is not possible to measure the length of tortuous arteries accurately during life.

It follows then that while from a much increased pulse wave velocity one can argue that the arterial walls have become less expansible, it cannot be said that the arterial walls are normal because the pulse wave velocity is numerically within normal limits.

As a rule the readings from two corresponding limbs in the same patient are in good accord, as can be seen from table 1. This relation was found to be disturbed in cases having aneurysm, as was also noted by Bramwell and Hill (3). In a case where there was clinical evidence of aneurysm the readings on the two arms varied widely:

R* TO BRACHIAL		BRACHIAL TO RADIAL		R* TO RADIAL	
Right	Left	Right	Left	Right	Left
8.90	6.75	3.34	2.36	5.60	3.82

* R wave of the electrocardiogram.

Conclusions in cases of arterio-sclerosis. Pulse wave velocity in arterio-sclerotic subjects may depend on two factors at least, ability of the vessel to expand (as determined by the character of the vessel wall and by the pressure conditions within it) and by tortuosity. The balance of these factors may be different in different vessels. In large vessels loss of expansibility produces the greatest effect, while in small peripheral vessels tortuosity may predominate. Tortuosity slows the actual transmission rate as well as altering the apparent rate through a wrong estimation of the vessel length.

Clinical application of measurement of pulse wave velocity in arterio-sclerosis would require a differentiation of these factors, possible perhaps by carefully noting the degree of tortuosity, and stiffening (as well as blood pressure) but such estimations are useless unless large and smaller vessels are differentiated and the opposing factors are recognized.

Records of aortic regurgitation. For these estimations varying types of cases were chosen. Of the fourteen patients examined eight were young adults having rheumatic lesions and no demonstrable sclerosis of the vessels while six had a positive Wassermann reaction and did have clinical evidence of damaged vessels. In some instances heart sound records were obtained and an attempt made to correlate the electrocardiographic and round records, in other cases apex beat records were taken. The sound secords were unsuitable for estimating the pulse wave velocity partly because of the large initial vibrations during rising tension time and partly because of complicating murmurs. The figures published from the expulsion period were determined from the apex beat record as described previously while for those calculated from the R wave of the electrocardiogram a correction was used of 0.03 second for the time from the peak of the R to the beginning of the expulsion period (1). The great variations in pressure in the vessels cause the curves to rise quickly so that the obtained records are easily and accurately read. Tables 6 and 7 give the results in detail, the data being grouped in accordance with the type of the lesion.

The averages of table 6 show that there was no conspicuous increase in pulse wave velocity in these cases as compared with the average rate in the normals of approximately the same age. On considering the average pulse wave velocity in table 7 it is seen that the rates are all above the average normal rates irrespective of age but when compared with the same age periods they agree closely. There was no evidence to indicate that the regurgitation was materially different in the two groups.

One may conclude therefore that aortic insufficiency *per se* has no effect on pulse wave velocity. If however there be changes in the vessel walls, producing loss of expansibility from advancing age or from syphilitic disease, then an increase of pulse wave velocity occurs.

Records of hyperthyroidism. In connection with another study (7),

eighteen examinations were made in six patients suffering from hyperthyroidism. In table 8 are collected the complete data for the series.

In these cases one observes that there is a greater variation than in normal individuals and this is probably to be expected since the vaso-

TABLE 6

Pulse wave velocity in rheumatic aortics

INITIAL	AGE	BLOOD PRESSURE				PULSE	ARM			LEG		
		Arm		Leg			A-R	A-Br.	Br.-R	A-DP	A-F	F-DP
M. H.	14	110/50	60	130/50	80	72	2.47*	4.44	2.47	4.30	3.29	5.46
R. W.	15	120/30	100			96	3.55*	3.31	4.11			
R. S.	16	120/58	62			96	4.55*	4.11	5.51			
B. T.	17	120/30	90	180/40	140	102	4.52*	3.80	6.65	4.27	3.79	6.20
McT.	21	110/48	62	130/44	86	49		3.19		4.25	3.29	5.28
A.	22	110/30	80	170/50	120	84	5.45*	4.52	5.15	5.94	4.27	8.10
D.	22	110/54	56	120/50	70	84	4.75*	3.83	6.68	4.92	3.81	6.11
E. B.	23											
7/12/23.		118/74	44			96	3.57	2.83		5.30	3.16	10.25
10/12/23.		118/74	44			82	2.97	2.67	4.32			
11/12/23.		98/64	34			85	3.19	2.81	5.38			
Average	19.6		63.2		99	84.6	3.24	3.44	5.01	5.30	3.59	7.34

* Denotes that the calculation is from the R wave rather than the apex beat.

TABLE 7

Pulse wave velocity in luetic aortics

INITIAL	AGE	BLOOD PRESSURE				PULSE RATE	ARM			LEG		
		Arm		Leg			A-R	A-Br.	Br.-R	A-DP	A-F	F-DP
W.	35	128/44	84	228/50	178	96	5.69*	4.57	9.04	6.27	4.38	8.87
A. S.	35											
4/12/23.		135/77	58			98	4.47	3.74	8.63			
5/12/23.		122/62	60			96	6.41	5.41	9.60			
6/12/23.		110/70	40			95	5.13	4.59	7.67	7.58	4.76	13.00
H.	45	126/58	68			77	5.20	4.24	12.32	6.45	4.50	12.45
A. P.	45	138/58	80	160/60	100	84	4.89	3.97	5.26	5.70	4.94	6.63
C. J.	49	154/50	104	290/68	122	60	4.75*	4.92	4.48	6.60	4.90	8.95
W. S.	56	146/52	94	210/80	130	84	5.48*	4.87	5.62	7.08	5.22	10.20
Average	44.1		73.5		142.5	86	5.30	4.71	7.67	7.02	5.68	9.47

* Denotes that the calculation is from the R wave rather than the apex beat.

motor control of these patients is so unstable. With constantly varying degrees of arterial contraction and relaxation changes of pulse wave velocity would occur. Comparing the records of the same patient on different occasions there seems to be an inverse relation between blood flow and

TABLE 8

Pulse wave velocity in hyperthyroidism

CASE NUMBER*	AGE	BLOOD PRESSURE		PULSE PRESSURE	PULSE RATE	A-Br.		Br.-R		A-R		A-F		F-DP		A-DP		AVERAGE A-R	CIRCULATION RATE PER LITER PER MINUTE	CALORIES PER SQUARE METER PER HOUR	REMARKS
		Systolic	Diastolic			Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left				
28/11/23																					
4. Female	20	112	70	42	90	3.83	4.70	8.55	8.46	5.34	6.50							5.92	4.70	55.5	Before operation
22/ 2/24		125	85	40	90	3.69	3.58	9.14	12.95	4.75	5.00	4.58	4.65	8.77	9.23	6.45	6.65	4.88	6.30	53.8	Before operation
26/ 2/24		125	85	40	92	—	3.00	—	5.75	—	3.52							3.52			Before operation
10/ 4/24		90	45	45	94	3.02	2.98	5.11	4.59	3.51	3.36	3.03		10.86		5.64		3.44	3.50	39.7	After operation
12. Female	18																				
24/ 3/24		128	90	38	140	3.79	4.06	11.48	11.48	5.27	5.50							5.35			Before operation
25/ 3/24		100	55	45	150	3.75	3.81	5.67	7.59	4.20	4.55	3.77		13.12		6.64		4.38	10.35	80.32	Before operation
28/ 3/24		130	85	45	138	4.95	5.18	7.30	6.79	5.54	5.59					7.00		5.57	8.46	68.34	Before operation
14. Female	31																				
17/12/23		137	98	39	115	3.57	4.05		11.10	4.72	5.00	3.72		14.57		6.82		4.86	7.60	56.6	Before operation
18/12/23		145	88	57	122	3.05	3.98	10.00	12.00	3.86	4.96	3.86		18.46		6.93		4.41	7.26	57.5	Before operation
9/ 4/24		145	100	45	96	5.38	6.07	4.89	5.37	5.55	5.92							5.75	4.42	50.10	After operation
15. Female	35																				
21/12/23		127	50	77	106	4.26	4.06	7.96	8.52	5.00	5.13	5.40		9.26		7.10		4.16	7.94	74.2	Before operation
20/12/23		122	58	64	120	3.58	3.50	5.83	5.11	4.06	3.86	4.17		8.07		5.74		3.96	10.00	87.9	Before operation
28/12/23		124	50	74	103	3.39	3.09	4.66	6.00	3.91	3.42	6.78		9.11		9.88		3.67	7.70	73.9	Before operation
31/12/23		129	65	64	101	3.31	3.38	9.48	5.41	4.15	3.87	3.54		13.13		6.17		4.01	7.66	65.9	Before operation
13. Male	30																				
24/ 3/24		145	67	78	100	4.22	4.00	10.40	7.65	5.16	4.77	3.64		13.63		6.31		4.97	11.54	100.6	Before operation
27/ 3/24		140	64	76	108	4.02	3.56	6.86	6.45	4.65	4.20	4.62		10.00		6.78		4.48	10.80	80.8	Before operation
16. Female	45																				
17/ 3/24		192	120	72	90	3.80	3.45	7.15	7.41	4.42	4.08	4.15		8.17		5.87		4.25	6.33	53.70	Before operation
20/ 3/24		190	135	55	100	3.77	3.50	8.80	9.14	4.61	4.30					6.57		4.45	7.76	52.3	Before operation
Average....	29.9	134	78.3	55.3	108.6	3.85	3.89	7.70	7.87	4.63	4.64	4.25		11.43		6.71		4.56	7.65	65.69	

* Case numbers correspond to those in the original publication (7).

TABLE 9

Correlating pulse wave velocity and circulation rate

CASE	PULSE WAVE VELOCITY IN METERS PER SECOND	CIRCULATION RATE LITERS PER MINUTE	REMARKS
1	4.47	6.09	Aortic insufficiency
	6.47	7.66	
	5.15	7.65	
2	3.53	6.45	Aortic insufficiency
	2.96	5.20	
	3.19	5.90	
3	4.86	7.60	Exophthalmic goiter
	4.41	7.20	
	4.75	2.76	
4	3.96	10.00	Exophthalmic goiter
	5.07	7.96	
	3.67	7.80	
	4.40	7.66	
5	4.00	5.16	Aortic insufficiency
6	3.60	5.70	Normal
7	4.68	5.83	Pernicious anemia. Hb. 20 per cent
8	4.25	6.33	Toxic adenoma
	4.76	7.76	
9	2.88	6.94	Secondary anemia; hemoglobin 60 per cent; diabetes
10	4.97	11.50	Exophthalmic goiter
	4.48	10.83	
11	4.38	10.35	Exophthalmic goiter
	5.57	8.47	
12	4.15	6.00	Exophthalmic goiter—post-operative
13	3.44	3.50	Exophthalmic goiter—post-operative
Average...	4.35	7.18	

pulse wave velocity; for instance, in case 4 where there was a blood flow of 4.70 liters per minute the velocity from apex to radial was 5.92 meters per second while with a blood flow of 6.30 liters per minute the velocity was

Case numbers correspond to those in the original publication (7).

reduced to 4.88 meters per second. Cases 12 and 15 show the same variation. While such a relation appears in instances with great variations in blood flow it does not appear where the blood flow variations are less marked. Finally the averages for table 8 are very close to the averages for normal individuals, the average age of the two groups being about the same. We may therefore conclude that pulse wave velocity within certain limits of cardio-vascular disturbance is unchanged in hyperthyroid disease.

Discussion. Because of the possible correlation between blood flow and pulse wave velocity a further series of cases was investigated to determine this point. Twenty-five examinations were made in thirteen patients (the circulation rates and pulse wave velocity being estimated at one sitting under the same conditions). The velocities from apex to radial in meters per second and the circulation rates in liters per minute are grouped in table 9. A statistical study was then made of these data. The average velocity apex to radial was 4.35 meters per second the standard deviation being 0.8564 and the coefficient of variation being 19.70. The average blood flow per minute was 7.18 liters, with a standard deviation of 1.932 and a coefficient of variation of 26.9. The correlation of pulse wave velocity to blood flow was 0.30 and the probable error is 0.125. From such a study it appears that the correlation holds in only about one-third of the cases.

As Bramwell (4) and Bramwell, Hill and McSwiney (3) have affirmed, there seems to be a relation between diastolic pressure and pulse wave velocity in normal individuals. To test this relation the numerical value was calculated for the various groups of cases examined. For the normals the following values were obtained:

	AVERAGE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY	CORRELATION	PROBABLE ERROR
Diastolic pressure in mm. Hg.....	72.5	18.37	25.3	0.723	0.12
Pulse wave velocity in meters per second, apex-radial.....	4.31	1.171	27.2		

The hyperthyroid group yielded a similar result.

	AVERAGE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY	CORRELATION	PROBABLE ERROR
Diastolic pressure in mm. Hg.....	78.3	7.755	9.9	0.81	0.055
Pulse wave velocity in meters per second, apex-radial.....	4.56	0.7223	16.0		

When making a study of the data for the aortic regurgitation cases (the two tables being combined) the following values were found:

	AVERAGE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY	CORRELATION	PROBABLE ERROR
Diastolic pressure in mm. Hg.....	54.4	10.03	29.5	0.12	0.16
Pulse wave velocity in meters per second, apex-radial.....	4.51	0.9317	20.6		

Similar treatment of the data from the cases of arterio-sclerosis also showed a very poor correlation, the actual values being:

	AVERAGE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY	CORRELATION	PROBABLE ERROR
Diastolic pressure in mm. Hg.....	104	21.85	21.0	0.044	0.125
Pulse wave velocity in meters per second, apex-radial.....	5.00	1.108	22.16		

It appears then that in cases where there are indications of changes in the cardio-vascular structure that the correlation which occurs in normals between diastolic blood pressure and pulse wave velocity is lost. This point will be discussed further in a study of the effects of adrenalin on pulse wave velocity.[2]

The correlation for systolic pressure was also calculated in several groups and the following values obtained:

	CORRELATION	PROBABLE ERROR
Normals.....	0.817	0.09
Hyperthyroids.....	0.110	0.16
Arterio-sclerosis.....	0.572	0.085
Adrenalin injection.....	0.850	0.035

It would seem then that factors such as hardening of the arterial wall and muscular contraction modify results in normal and abnormal subjects so that diastolic pressure is not always the dominant factor. From these figures it would appear that in normal subjects pulse wave velocity correlates about equally well with systolic or diastolic pressure, in hyperthyroid disease there is a relation of pulse wave velocity to diastolic pressure but not to systolic, while in arterio-sclerosis (and after adrenalin) there is no relation to diastolic pressure but a close relation to systolic pressure. It should be remembered that the statistical methods have been applied

[2] To be published in a later paper.

to relatively small groups of figures and therefore should be accepted provisionally.

GENERAL SUMMARY AND CONCLUSIONS

1. Pulse wave velocity findings in a series of 51 normal individuals between the ages of 16 and 41 are reported, the average velocity from apex to radial being 4.90 meters per second and that from apex to dorsalis pedis being 5.61 meters per second.

2. The statement made by Bramwell (4) and Bramwell, Hill and McSwiney (3) that pulse wave velocity, in normal individuals varies with diastolic pressure is confirmed and the numerical correlation found in a series of 8 cases was 0.723 with a probable error of 0.12.

3. Data from a series of 30 cases of arterio-sclerosis are presented showing that in some instances the velocity of the pulse wave is increased and in others is decreased.

4. Findings obtained from a circulation schema show that wave velocity varies in accordance with the formula developed by Moens. In addition tortuosity slows the velocity while changes of the rate of impulse have no demonstrable effect.

5. It is suggested that varying combinations of these forces are accountable for the two types of response in arterio-sclerosis.

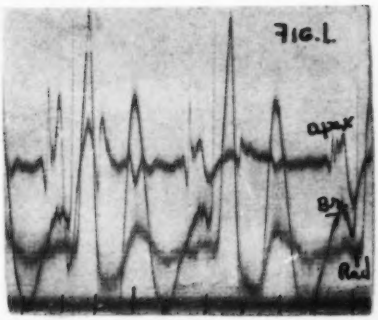
6. From a statistical study of the data obtained in cases of arterio-sclerosis, it appears that there is no correlation between diastolic blood pressure and pulse wave velocity but that there is a relation to systolic blood pressure.

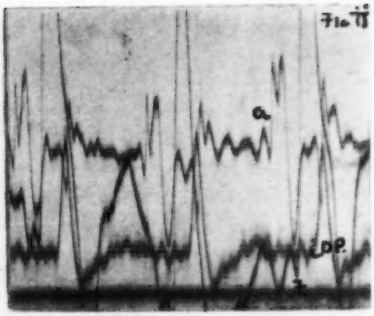
7. Study of fourteen cases of aortic regurgitation shows that with this lesion pulse wave velocity does not change and that there is no correlation between pulse wave velocity and diastolic blood pressure.

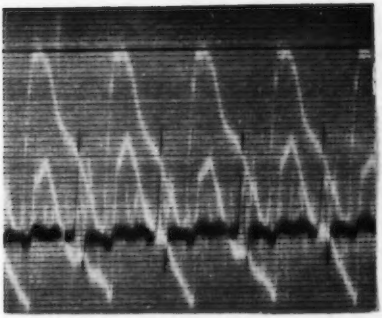
8. From a study of 6 cases of hyperthyroidism it seems that this disease does not materially alter pulse wave velocity. In these cases there was a correlation of 0.81 between pulse wave velocity and diastolic blood pressure with a probable error of 0.055.

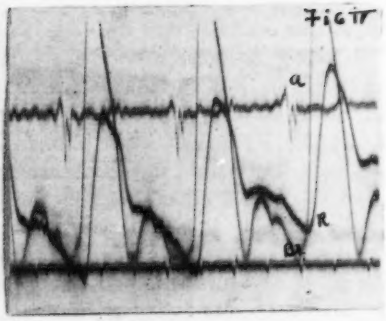
BIBLIOGRAPHY

(1) Sands: This Journal, 1923, lxvii, 203.
(2) Matzke, Priestley and Sands: This Journal, 1923, lxvii, 216.
(3) Bramwell, Hill and McSwiney: Heart, 1923, x, 248.
(4) Bramwell: Quart. Journ. Med., 1924, xvii, 225.
(5) Bazett and Dreyer: This Journal, 1922, lxiii, 94.
(6) Dickson: Journ. Physiol., 1922, lvi, Proc. Physiol. Soc., xl.
(7) Davies, Meakins and Sands: Heart (in press).
(8) Moens: Die Pulskurve. Leiden, 1878, p. 90.









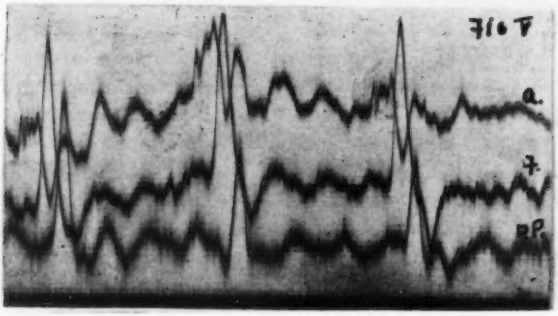


Fig. 1. Case 2. Table 1. Apex, brachial, radial. Normal. Time $\frac{1}{5}$ second in all records.

Fig. 2. Case 2. Table 1. Apex, femoral, dorsalis pedis. Normal.

Fig. 3. Case 2. Table 5. Rheumatic aortic lesion. Brachial, electrocardiogram lead II, radial.

Fig. 4. Case 11. Table 3. Arterio-sclerosis with relatively slow conduction. Apex, brachial, radial.

Fig. 5. Case 24. Table 4. Arterio-sclerosis with somewhat increased conduction. Apex, femoral, dorsalis pedis.

STUDIES IN PULSE WAVE VELOCITY

IV. Effect of Adrenalin on Pulse Wave Velocity

D. MURRAY LYON and JANE SANDS[1]

From the Department of Therapeutics, University of Edinburgh

Received for publication October 29, 1924

In studying pulse wave velocity in abnormal subjects, it is important to determine how far this velocity may be affected by temporary changes in the vessel wall, which may occur unequally in different patients. Such a temporary change may be induced by adrenalin, and it seemed therefore desirable to test the effect of this drug on different patients and to compare the results with those obtained in the vascular reactions produced by local application of heat or cold.

General methods. Patients were examined after a preliminary period of rest during which time repeated observations of blood pressure and pulse wave velocity were taken in order to make sure that a steady level had been attained. Optical records of pulsations were taken from the brachial and radial arteries and the apex beat, the tambours being unchanged throughout the whole experiment, and the term pulse wave velocity is here used for the average velocity estimated by comparison of the apex beat and radial records. The general technic used was that previously described (1). Estimations of circulation rate were made by the gasometric method described by Davies and Meakins (2). The air for estimation of the arterial carbon dioxide tension was collected in vacuum tubes, while the expired air was collected during minute periods in a Douglas bag. The samples of air for estimating venous carbon dioxide were directly examined on a Haldane gas analysis apparatus. Readings of systolic and diastolic blood pressure were made by the auscultatory method every two minutes and the heart rate was recorded at the same intervals. The times of taking pulse records and gas samples were largely controlled by these readings. Adrenalin chloride solution (1–1000) P. D. & Co. was employed in quantities of 0.5 to 1.0 cc., given subcutaneously. The subjects examined included both males and females of varying ages who were suffering from diseases such as are found in a general medical ward.

[1] Fellow of the Medical Board, National Research Council, U. S. A.

GENERAL RESULTS FOLLOWING ADMINISTRATION OF ADRENALIN. The findings corresponded closely with those already described (3) and included changes in systolic and diastolic blood pressure, pulse rate, metabolism and circulation rate, as well as an alteration in the rate of conduction of the pulse wave.

All cases showed a primary increase in systolic blood pressure followed by a slower decline to the initial level. The diastolic pressures followed no constant rule, being sometimes raised, sometimes lowered, and occasionally raised and lowered in different periods of the reaction. In all cases an increase in pulse pressure was also observed.

Cardiac acceleration occurred in all the subjects examined, but varied considerably in degree. The increase in rate, as a rule, came on more slowly than the other changes. In five cases on whom the metabolic

TABLE 1

Pulse wave velocity (apex-radial) following the injection of adrenalin

CASE NUMBER	AGE	TIME AFTER INJECTION											
		0 minute	1 minute	2 minutes	3 minutes	4 minutes	5–10 minutes	11–20 minutes	21–30 minutes	31–40 minutes	41–50 minutes	51–60 minutes	1 hour 10 minutes
2	21	5.37			5.75			5.66	7.06	7.10			6.48
3	27	4.63				5.44	5.51	5.05	6.00			5.17	4.90
4	47	3.88		3.93		3.91	4.11	4.64	5.35			4.54	
5	37	4.85		8.23		7.74	7.56	7.46		8.35			
6	23	3.9		4.5	4.6			4.6	5.3	5.0			
7	20	3.52	3.77	3.79	4.22	4.02	3.95	3.92					
8	18	5.50	5.50	5.93	6.07	5.84	5.98	5.43					
9	30	4.28	4.45	4.31			4.38	4.81	4.70	5.20			
10	45	3.90	4.64	4.56		4.56	4.56	4.63	4.63	4.40			

rate was estimated it was found to be increased, which confirms the findings in a previous communication (3). The disturbances of metabolism were found to follow closely the changes in the systolic pressure and circulation rate (subjects 1 and 2, figs. 1 and 2). Similar changes were observed in the circulation rates as expressed in liters per minute thus corresponding to the finding of Davies, Meakins and Sands (4) that changes of circulation rate vary directly with metabolic rate. An increase of pulse wave velocity occurred in every instance during the circulatory disturbance following the adrenalin injection. Certain other phenomena were noticed; tremor occurred practically in every case, facial pallor was frequent and was sometimes followed by flushing. In three cases of exophthalmic goiter a rather severe reaction took place in which there was nausea and vomiting, coming on after the height of the circulatory disturbance.

FIG 1.

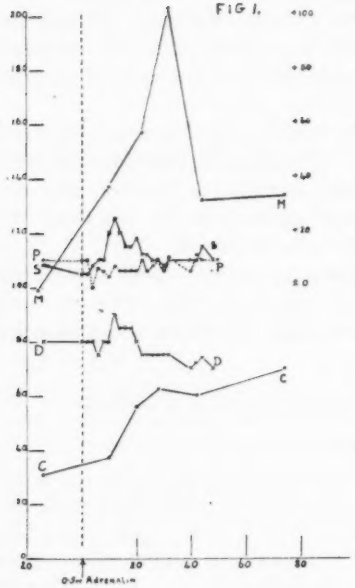


FIG 2.

FIG 3.

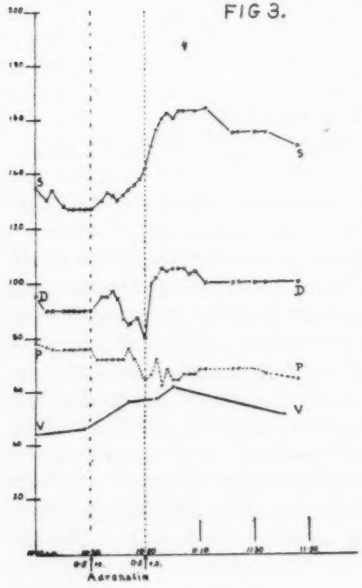
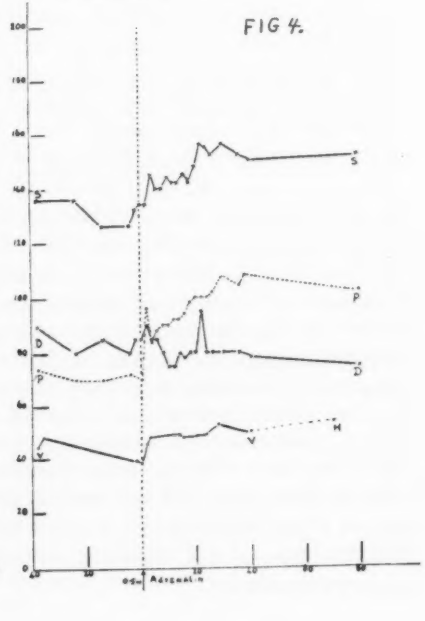
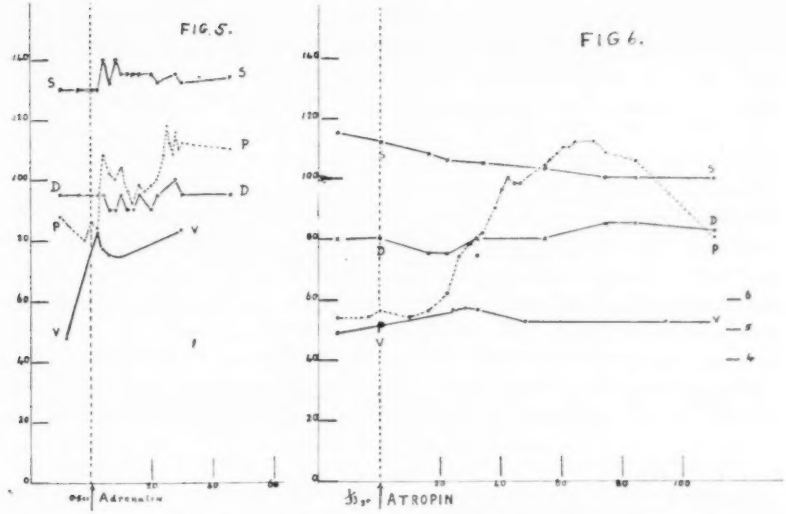


FIG 4.





In all instances M = metabolic rate; S = systolic blood pressure; D = diastolic blood pressure; P = pulse rate; C = circulation rate, and V = pulse wave velocity. In the case of pulse wave velocity the scale reading is to be divided by 10.

Fig. 1. Showing changes in systolic and diastolic blood pressure, metabolism and circulation rate following an injection of adrenalin.

Fig. 2. Showing same circulatory changes as figure 1. Subject 2. Note increase of circulation rate.

Fig. 3. Subject 2 on following day. Note the increase in pulse wave velocity.

Fig. 4. An instance where the curve of pulse wave velocity could be said to follow either that of systolic or diastolic pressure change.

Fig. 5. An instance where the pulse wave velocity curve appears to follow the pulse rate curve.

Fig. 6. After injection of atropin. No correlation between pulse wave velocity and pulse rate.

In a general way all the changes observed (with the exception of the pulse rate and the diastolic blood pressure) appeared to run more or less parallel, so that they may be considered as components of a single reaction. This reaction came on with precipitate suddenness in a few cases reaching the maximum values within the first five minutes, in others the changes occurred more slowly so that the highest values might be delayed for twenty or thirty minutes.

CORRELATION OF PULSE WAVE VELOCITY WITH OTHER DATA. In table 1 the pulse wave velocity readings are arranged according to the time after the adrenalin administration. Though there is no case that does not show an increased velocity, the time this change occurs varies greatly,

TABLE 2

The effect of adrenalin on blood pressure, pulse rate and pulse wave velocity

CASE NUMBER	SEX	AGE	SYSTOLIC BLOOD PRESSURE	DIASTOLIC BLOOD PRESSURE	PULSE RATE	PULSE WAVE VELOCITY A–R	PULSE PRESSURE	PULSE PRESSURE × PULSE RATE	REMARKS	Date
2	Male*	21	135	95	78	4.5†	40	3,120	Diabetes. No circulatory disturbance	7/1/24
			162	114	68	6.1†	48	3,264		
			150	110	64	5.1†	40	2,560		
3	Male	27	130	90	66	4.7	40	2,640	Gastric ulcer	13/2/24
			162	85	84	6.1	77	6,468		
			145	85	72	5.2	60	4,320		
4	Male	47	125	95	62	3.9	30	1,860	Biliary calculi Jaundice	
			135	100	64	5.3	35	2,240		
			125	95	60	4.7	30	1,800		
5	Female	37	130	95	86	4.9	35	3,010	Diarrhea	28/2/24
			132	95	108	8.3	37	3,996		
			132	95	110	7.5	37	4,070		
6	Female	23	134	85	70	3.9	49	3,430	Nervous exhaustion	22/2/24
			155	80	100	5.3	75	7,500		
			138	90	74	4.7	48	3,552		
7	Female	20	124	95	100	3.5	29	2,900	Exophthalmic goiter	26/2/24
			140	90	112	4.3	50	5,600		
			140	70	106	3.9	70	7,420		
8	Female	18	128	85	148	5.3	43	6,364	Exophthalmic goiter	24/3/24
			142	85	156	6.1	57	8,892		
			120	70	160	5.5	50	8,000		
9	Male	30	180	80	100	4.1	100	10,000	Exophthalmic goiter	25/3/24
			130	40	108	4.4	90	9,720		
			154	65	96	4.8	89	8,544		
			165	22	104	5.2	143	14,872		
10	Female	45	185	125	70	3.9	60	4,200	Toxic adenoma. Cardiac enlargement. Chronic nephritis. Hypertension	18/3/24
			184	90	98	4.9	94	9,212		
			172	115	80	4.4	57	4,560		
Average.....		29.8	145	87	93	5.18	58	5,504		

* Subjects 1 and 2—the data recorded are circulation rate and not pulse wave velocity.

† The three readings in each case represent the data obtained with the lowest, medium and highest pulse wave velocity.

as did the other reactions observed (see figs. 1 to 6). By inspection of these graphs it is not possible to decide whether the pulse wave velocity varies directly with the systolic pressure, pulse pressure, or pulse rate. This question has therefore been investigated by treating the data statistically.

STATISTICAL STUDY. For this purpose it was considered sufficient to choose three points from the graph of each patient examined, including the lowest, highest and medium values of pulse wave velocity together with the readings of systolic and diastolic blood pressure and pulse rate which occurred at times corresponding to these three values. The data are grouped in table 2. The average values are systolic blood pressure 145 mm. Hg, diastolic blood pressure 87 mm. Hg, pulse pressure 58 mm. Hg, pulse rate 93 per minute, pulse rate multiplied by pulse pressure 5504 and pulse wave velocity (apex to radial) 4.18 meters per second.

The standard deviations and coefficients of variation are as follows:

	STANDARD DEVIATION	COEFFICIENT OF VARIATION	CORRELATION TO PULSE WAVE VELOCITY	PROBABLE ERROR
Systolic blood pressure	18.76	13.0	0.82	0.035
Diastolic blood pressure	20.49	23.5	0.346	0.11
Pulse pressure	26.08	45.0	0.865	0.030
Pulse rate	27.07	29.0	0.262	0.12
Pulse pressure × pulse rate	1,897.0	34.4	0.047	
Pulse wave velocity	1.077	20.8		

That the standard deviations and coefficients of variation should be relatively great is to be expected from the nature of the experiments since great disturbances of the various factors concerned were produced. The correlation of diastolic blood pressure to pulse wave velocity is very low and entirely changed from the more common relation found in the previous study (5). There is no relation between pulse wave velocity and the product of pulse rate and by pulse pressure, which has been used as an estimate of cardiac activity. In another paper (4) it has been shown that there is no correlation between this product and circulation rate and from the correlation value obtained here it seems that there is no relation to pulse wave velocity either. The changes in diastolic pressure were relatively small and it follows that any change in pulse pressure would depend mainly on changes in systolic pressure. Thus one finds about equally high correlations for pulse pressure and systolic pressure. The correlation for systolic pressure is high and the probable error small so that it would seem that pulse wave velocity in this series of cases varies with systolic blood pressure, and pulse pressure, which also vary together.

In subject 2 the effect of adrenalin injection on the circulation rate and metabolism was estimated and on the following day the changes produced by such an injection on blood pressure and pulse wave velocity were determined for comparison (see figs. 2 and 3). It is clear that metabolism, circulation rate and pulse wave velocity all increase in a similar fashion.

COMPARISON OF EFFECTS WITH THOSE PRODUCED IN OTHER WAYS: *Atropin injection.* Judging by figure 4 it might seem that the changes in pulse wave velocity were associated with changes in blood pressure, while from figure 5 it seemed possible that the pulse rate had some influence on the conduction of the pulse wave. It was therefore thought that the reaction of the patient to atropin might help to determine whether this were true. A large dose of atropin sulphate (gr. $\frac{1}{33}$) was given subcutaneously to two subjects, one having marked jaundice with bradycardia, and the other diabetes and also a slow pulse (fig. 6 shows the type of result). Conspicuous changes in pulse rate occurred in both cases unaccompanied by any pronounced alterations in blood pressure. Such changes of blood pressure as did occur might be explained as the result of the prolonged inactivity of the subject. The pulse wave velocity in one instance was unchanged and in the other was only slightly changed, but not following the curve of pulse rate. This therefore confirms the deduction from the statistical study of the adrenalin reactions.

Local changes in temperature. The patients were seated with both arms resting on a table on a level with the heart and records of apex beat and the brachial and radial pulse on one arm were taken. A preliminary period of rest was given as usual and after basal records had been taken heat was applied to the arm and hand by means of a large electric hot-air bath, the tambours being unchanged throughout the experiment. From table 3 it is seen that heat caused a slowing of the pulse wave and this regardless of whether the heat was applied to the arm from which the curves were being taken or to the opposite arm. The one case failing to show this reaction was no. 10.

After the effect of heat had been tried on the subject, the arm was packed in cracked ice, the tambours being untouched. The rate immediately rose from 4.70 to 6.7 meters per second, a figure well above the original basal values. In case 10 cold had as little effect as heat and it is also interesting to note that even under adrenalin the rate was only slightly raised, increasing from 3.9 to 4.9 meters per second. Pertinent to the discussion of arterio-sclerosis in a previous paper (5) this case is an instance of a rate slower than would be expected in a person aged 45, who had a blood pressure considerably above normal and where arterial disease was clinically demonstrable. The probable explanation seems to be that the vessels were so diseased as to be unable to react to changes in environment. We assume that the effect of heat is to cause vascular relaxation,

and the observed changes in pulse wave velocity were so great that they are again probably not simply dependent on blood pressure changes.

Discussion. It would appear from these results that variations may occur in the wall of a vessel, particularly in the muscular coat, which may alter considerably the distensibility of the vessel and so change the pulse wave velocity and may do so without necessarily changing the diastolic pressure. While under normal conditions the distensibility of the vessel is largely dependent on the diastolic pressure, as has been suggested by Bramwell and Hill, so that the pulse wave velocity is definitely correlated with this pressure, this is no longer the case under these conditions and the correlation is now with systolic pressure. Such a result suggests a considerable decrease in the distensibility of the main vessel trunks, since such a change in the vessel would tend to increase systolic and decrease diastolic pressure while increasing the velocity of wave

TABLE 3

Effect of heat on pulse wave velocity

CASE NUMBER	HEAT ON SAME SIDE			HEAT ON OPPOSITE SIDE	
	Basal	Effect of heat	Temperature	Basal	Effect of heat
			°C.		
10	3.92	3.92	57	4.27	4.20
11	5.21	4.70	57		
12	5.26	4.26	42	5.35	3.89
		4.31	57		
		3.84			
13	7.41	4.69	51	5.04	4.71

transmission. The few experiments recorded on the effect of local temperature also support the hypothesis that the condition of the muscular coat of the vessels is variable, and a factor by no means negligible even in the larger vessels, though the evidence is here incomplete.

SUMMARY AND CONCLUSIONS

1. It has been shown in the present series of cases that the circulatory disturbance following the administration of adrenalin includes not only alterations in the blood pressure and pulse rate but also an increase in the circulation rate and in pulse wave velocity, these changes being uniformly found in all subjects examined.

2. An attempt to correlate the increase in pulse wave velocity with the various other reactions produced has shown the close relationship with

the systolic blood pressure (and also the pulse pressure). Diastolic blood pressure, pulse rate, and pulse pressure multipied by pulse rate, show little or no correlation.[2]

3. The effect on pulse wave velocity of increased pulse rate under atropin has also been studied and no relationship between these has been found.

4. In a few cases examined the application of dry heat to the limb had the effect of slowing the pulse wave velocity.

BIBLIOGRAPHY

(1) SANDS: This Journal, 1923, lxvii, 203.
(2) DAVIES AND MEAKINS: Heart, 1922, ix, 191.
(3) LYON: Quart. Journ. Med., 1923, xvii, 19.
(4) DAVIES AND MEAKINS AND SANDS: Heart (in press).
(5) SANDS: This Journal, 1925, lxxi, 519.

[2] The factors concerned in influencing pulse wave velocity are being further investigated and will be discussed in a future paper.

THE RELATION OF CALCIUM RESTRICTION TO THE HATCHABILITY OF EGGS

G. DAVIS BUCKNER, J. HOLMES MARTIN AND A. M. PETER

From the Kentucky Agricultural Experiment Station, Lexington

Received for publication November 20, 1924

Under ordinary conditions approximately 20 to 25 per cent of the fertile eggs incubated do not hatch and approximately 5 to 10 per cent of the chicks hatched are classed as weaklings that should be killed for economic reasons.

The object of this investigation, a project of this Experiment Station, was to determine whether this low viability is caused in part by a deficiency in the supply of calcium reaching the embryonic chick or influencing the metabolism of the embryonic development.

Riddle (1) has published very interesting results concerning inadequate eggshells and the early death of embryos in the egg, in which he has observed the hatchability of eggs laid under apparently normal conditions, by ringdoves having a normal diet, and has determined the inadequate shells by the rate of evaporation of moisture from the eggs. He concludes, among other things, that "the production of inadequate shells and the early death of the embryos are thus closely associated, although the relative inadequacy of a particular shell is but loosely correlated with the death of the particular embryo contained within it. An unknown and more deeply seated cause is responsible for both the occasional inadequate or irregular shells and the numerous early deaths of embryos."

In another paper Riddle and Hanke (2) give results concerning the effect of feeding calcium lactate and lactophosphate upon reproductive secretions of ringdoves and upon the total inorganic constituents of the eggshell. They conclude in part that "The amount of inorganic substance laid down in the eggshell was practically unchanged by the extra calcium feeding."

It is obvious that in the above experiments Riddle and Hanke were dealing with pigeons whose ability to lay eggs having thin or inadequate shells was not influenced by the addition of the calcium compounds mentioned, to the diet, and that this condition was peculiar to the birds in question. In other words, these were normally produced inadequate shells and some of the eggs with inadequate shells hatched. The authors finally conclude that "The production of inadequate shells, or of thin shelled eggs,

which is associated with the early death of many bird embryos is probably not caused by an inadequate calcium supply in the food."

Our experiments (3) have shown that calcium limitation in a ration for hens does not alter the amount of the calcium in the liquid portion of the eggs produced but that it does cause thin shells to be formed.

Delezenne and Fourneau (4) have shown that approximately 75 per cent of the calcium of the mature chick embryo comes from the shell during the process of incubation.

What then is the comparative hatchability of the eggs having normal thick shell and those having thin shell produced by calcium deficiency, when all other factors are equal? It seems reasonable to believe that there must be a difference when we know that the normal dry eggshell of the average white Leghorn hen weighs 5.2 grams as compared to 3.5 grams for the average egg from the same breed under calcium limitation.

It is generally accepted as true that close inbreeding and confinement with restriction of green food during the winter months diminish materially the hatchability of eggs. It is further believed by some that low hatchability is associated, in a measure, with high egg-production and it may be that this is caused by the thinning of the eggshell, which we know occurs during high egg production.

In our experiment 3 lots of hens were used, each containing 10 single-comb white Leghorns which came from the same parent stock and were the same age. These hens had been raised under identical conditions and each lot had an average trap-nest record of approximately 160 eggs per hen, for their pullet year. On November 1, 1923, the date when this experiment commenced, each lot was put into a house 10 $\times$ 12 feet, all houses being identical in construction and orientation. Lot 1 was allowed daily freedom of a grass range while lots 2 and 3 were kept in their houses throughout the experiment. All three lots were fed *ad libitum* the same ration, which consisted of corn, wheat and buttermilk, and were supplied with cabbage, lettuce or other green foods twice a week during the experiment. Lots 1 and 2 received oystershell *ad libitum* while lot 3 received no calcium supplement to the foods mentioned. Grit, which had been freed of calcium carbonate or other soluble calcium compound by washing with hydrochloric acid and water, was supplied at the rate of 20 grams per month per hen, to lots 2 and 3, while lot 1 received it *ad libitum*. The oat straw litter was changed when necessary. Three cockerels of the same age, coming from the same parent stock and whose fertilizing powers were known to be high, as nearly equal as possible, were placed in the 3 pens.

Starting March 1, 1924, after the hens had been under the above described conditions for 4 months, the eggs were collected in 14-day periods for incubation. At this time the constant average dry weights of the eggshells for lots 1, 2 and 3 were, respectively, 5.2, 5.4 and 3.5 grams.

The eggs were examined for soundness, placed in a Newtown Giant incubator and on the 18th day examined for fertility and dead germs. Sufficient time was allowed after the 21st day for those late in hatching, and those remaining unhatched were classed as dead in the shell. The results are shown in table 1. Always more eggs were laid in lot 1 than in the other lots, presumably owing to the freedom of the grass range. As has been noted, the lack of calcium causes a smaller number of eggs in the lot receiving no calcium supplement, than in the corresponding lot which received oyster shell.

It will be seen that for lot 1 the hatchability is nearly the same for the first two months, with an unaccountable fall for the third hatch. In lot 2 the hatchability rises for the third hatch, while in lot 3 which received no

TABLE 1

NUMBER OF HATCH	LOT NUMBER	DATE OF SETTING	TOTAL NUMBER OF EGGS INCUBATED	NUMBER OF INFERTILE EGGS	NUMBER OF DEAD GERMS BEFORE 18TH DAY	NUMBER OF DEAD IN SHELL	NUMBER HATCHED	PER CENT INFERTILE	PER CENT DEAD GERMS BEFORE 18TH DAY	PER CENT DEAD IN SHELL	PER CENT HATCHED
1	1, shell, range	March 14/24	76	8	6	12	50	10.5	7.9	15.8	65.8
	2, shell, no range	March 14/24	69	11	11	19	28	16.0	16.0	27.5	40.6
	3, no shell or range	March 14/24	19	1	6	8	4	5.3	31.6	42.1	21.0
2	1, shell, range	March 28/24	58	7	7	7	37	12.1	12.1	12.1	63.8
	2, shell, no range	March 28/24	42	3	9	15	15	7.1	21.4	35.7	35.7
	3, no shell or range	March 28/24	17	2	5	7	3	11.8	29.4	41.2	17.7
3	1, shell, range	April 11/24	78	11	14	19	34	14.1	17.9	24.4	43.6
	2, shell, no range	April 11/24	59	0	12	13	34	0	20.3	22.0	57.6
	3, no shell or range	April 11/24	18	3	11	4	0	16.7	61.1	22.2	0

calcium supplement there was a fall from 21.0 per cent in the first hatch to 17.0 per cent in the second hatch which was followed by 0 per cent in the third hatch. It is interesting to note that in lot 3 the largest per cent of deaths of embryos occurred from the 18th to the 21st day in the first and second hatch but then in the third hatch, the largest per cent was prior to the 18th day, the deaths being earlier as the season advanced, under these conditions.

On May 1 oyster shell was added to the ration of lot 3 and withheld from lots 1 and 2, all other conditions remaining unchanged. Three settings were then made, starting May 12, May 27 and June 10, the results of which are given in table 2.

Here we see an appreciable decrease in the number of eggs laid by lots

1 and 2 and an increase in lot 3 which is directly influenced by the supplementary calcium.

The decrease in the percentages of chicks hatched in lots 1 and 2 for these three hatches also is interesting. This is particularly noticeable in lot 2 which did not have the range from which to obtain a supply of calcium. On the other hand, we have a decided and continued increase in the hatchability of the eggs in lot 3 which now received the supplementary calcium carbonate and the larger percentage of deaths of embryos occurred subsequent to the 18th day. Withholding the calcium supplement from lot 1 seems to have caused a temporary decrease in the fertility of the eggs of this lot, but there is a marked decrease in the fertility of the eggs of lot 2 following the withholding of the supplemental calcium carbonate.

TABLE 2

NUMBER OF HATCH	LOT NUMBER	DATE OF SETTING	TOTAL NUMBER OF EGGS INCUBATED	NUMBER OF INFERTILE EGGS	NUMBER OF DEAD GERMS BEFORE 18TH DAY	NUMBER OF DEAD IN SHELL	NUMBER HATCHED	PER CENT INFERTILE	PER CENT DEAD GERMS BEFORE 18TH DAY	PER CENT DEAD IN SHELL	PER CENT HATCHED
4	1, no shell, range	May 12/24	46	9	10	17	10	19.6	21.7	37.0	21.7
	2, no shell, no range	May 12/24	37	14	9	10	4	37.8	24.3	27.0	10.8
	3, shell, no range	May 12/24	29	6	6	11	6	20.7	20.7	37.9	20.7
5	1, no shell, range	May 27/24	44	3	4	29	8	6.8	9.1	65.9	18.2
	2, no shell, no range	May 27/24	33	11	6	11	5	33.3	18.2	33.3	15.2
	3, shell, no range	May 27/24	40	4	5	18	13	10.0	12.5	45.0	32.5
6	1, no shell, range	June 10/24	43	3	9	13	18	7.0	21.0	30.2	41.9
	2, no shell, no range	June 10/24	22	8	8	5	1	36.4	36.4	22.7	4.6
	3, shell, no range	June 10/24	36	4	4	15	13	11.1	11.1	41.7	36.1

From the foregoing experiment it seems that the hatchability of eggs is markedly diminished by withholding a sufficient supply of calcium carbonate from the ration of laying hens. In what way this deleterious action is produced we cannot say. However, we can say that some of the possible factors causing this condition are the unnatural carbon dioxid-oxygen exchange, the increased rapidity of evaporation of water caused by thin shells or some metabolic process which is dependent on the supply of calcium. The possibility of any vitamine factor or any factor other than the lack of calcium is excluded from effecting these anomalies because of a reversal of results when the calcium supplement was added or withheld. This also eliminates any other deep-seated cause such as must have influenced Riddle's results.

The striking character of these results seems to the authors worthy of a verification which will be made at the appropriate time.

BIBLIOGRAPHY

(1) RIDDLE: This Journal, 1921, lvii, 250.
(2) RIDDLE AND HANKE: This Journal, 1921, lvii, 264.
(3) BUCKNER AND MARTIN: Journ. Biol. Chem., xli, 195.
(4) DELEZENNE ET FOURNEAU: Annales de l'Institute Pasteur, xxxii, no. 9.

ATTEMPTS TO PRODUCE EXPERIMENTAL INCREASE IN THE RATE OF OUTPUT OF THYROGLOBULIN BY THE THYROID GLAND

A. J. CARLSON, L. HEKTOEN AND R. SCHULHOF

From the Hull Physiological Laboratory of the University of Chicago and the John McCormick Institute for Infectious Diseases

Received for publication November 17, 1924

Thyroglobulin, prepared according to the Ostwald method, and injected into rabbits, leads to appearance in the rabbits' plasma of a specific precipitin for thyroglobulin (4). By means of this specific precipitin reaction it has been shown that thyroglobulin is present in demonstrable quantities in the lymph of the thyroid glands, and in lymph of the common neck lymphatics, but it is not present in demonstrable quantities in lymph from the thoracic duct or in arterial blood (5). These observations were made on dogs with enlarged thyroids.

The reliability of the precipitin test for thyroglobulin in lymph and blood is indicated by the following typical experiment.

The lymph from the left side of dog 11, taken after the injection of pilocarpin, was diluted in 5 parts of salt solution and mixed with an equal quantity of antiserum against dog serum, the precipitate being removed by centrifugation. This was done in order to remove the ordinary proteins in the lymph. After being treated in this way the lymph did not react with serum against dog serum, but it still reacted in fairly high dilutions (640) with antidogthyroglobulin serum 1021.

We have now extended our studies to thyroid vein blood, and attempted to induce increased output of thyroglobulin from the glands by various experimental procedures, such as massage of the thyroid, stimulation of the nerves to the thyroid, and the administration of drugs that stimulate secretory nerve mechanisms or otherwise augment gland secretions.

The question of control of thyroid activity by secretory nerves, because of its physiological and practical importance, has stimulated much experimental work. A considerable part of the data is contradictory, and most of the experimental methods have been indirect, so that the results, even when positive, may be capable of more than one interpretation. The only points that appear clearly established are: *a*, the thyroids receive nerve fibers from the cervical sympathetics that pass both to the blood vessels and to the gland cells. The former are vasomotor

in function, the latter may be secretory or visceral afferents; *b*, complete denervation of the thyroid glands does not induce any demonstrable interference with thyroid function. The most recent striking evidence on this point is that reported by Willier (8). This investigator transplanted chicken thyroid into the chorio-allantoic membrane of chick embryos. These grafts became vascularized and induced symptoms of hyperthyroidism. There are no nerves in the chorio-allantoic membrane. But the fact that thyroid grafts, or the denervated thyroids, can function normally does not tell us what the thyroid secretory nerves may do, if and when they are present.

The influence of the thyroid nerves or the thyroid nerve stimulation on the iodine in the thyroid gland is uncertain. The recent careful work of Van Dyke (7) on this point failed to reveal any demonstrable effect of thyroid nerve stimulation on the iodine concentration. But even if such procedures altered the iodine concentration in the gland, this might be due to the vascular changes produced by the stimulation. The work of Asher showing that the stimulation of the thyroid nerves sensitizes the depressor nerve has not been confirmed, and according to the recent report of Csillag (9) the alleged synergistic action of the thyroid hormone on the action of adrenalin cannot be used as a test for the thyroid hormone in the blood.

Cannon and his collaborators have reported that stimulation of the thyroid nerves induces a temporary change in electrical tension in the gland (2), and stimulation of these nerves or direct massage of the thyroid glands accelerates the denervated heart (3). Both of these results are interpreted by Cannon as increased secretory activity or increased hormone output, and hence as evidence of secretory nerve control of the thyroids. In our judgment the active vascular changes induced in the thyroids on stimulation of the thyroid nerves have not been eliminated as a probable factor in electrical changes in the gland. The acceleration of the denervated heart by thyroid nerve stimulation is more suggestive, except for the prompt appearance and short duration of the phenomena. Thyroid or thyroxin administration seems to have a much longer latent period and longer action, at least on the basal metabolic rate. Cannon also reports that the thyroid nerve stimulation is without effect when the blood pressure is low, as well as when the pulse rate is high, and the experiment yielded only negative results in March and April. This last fact seems particularly puzzling, since there is as yet no evidence of seasonal hypofunction of the thyroid glands in a carnivorous animal like the cat, especially in a non-goitrous region like Boston. Direct massage (by trauma) and marked vasoconstriction in a gland (by asphyxia) may lead to discharge into the blood of cardio-vascular stimulants other than the specific gland hormones, as has been shown by Cannon

for the liver. Rogoff collected blood from the thyroid vein before and after massage of the thyroids or stimulation of the thyroid nerves, and applied the tadpole feeding test to this blood, as well as direct iodine determinations. Both series of tests were negative, except in the case of one animal.

The present series of experiments was done on 17 dogs. All of the dogs had varying degrees of enlargement of the thyroids. Light ether anesthesia was used.

Massage of the thyroid glands was made by hand manipulation through the skin. The cervical sympathetic trunk was separated from the vagus, sectioned near the stellate ganglion and stimulated with a weak tetanizing current for one-minute periods alternating with one minute rest. A strength of stimulus was selected that caused definite dilatation of the pupil. In this way the thyroid nerves were stimulated for periods varying from 30 to 60 minutes.

Thyroglobulin in thyroid vein blood. Under the conditions of our experiments the blood from the thyroid vein of the dogs gave a precipitin reaction with the thyroglobulin antiserum. There is one exception to this (dog 23). In this animal we also failed to secure evidence of the presence of thyroglobulin in the neck lymph. On the basis of these tests the concentration of the thyroglobulin in the thyroid vein blood is about the same as in the thyroid lymph. Occasionally the concentration appeared higher in the blood. At no time could we detect the presence of thyroglobulin in arterial blood, or in thoracic lymph, or lymph from the leg. This confirms our earlier findings. The failure to secure the reaction for thyroglobulin in arterial blood may be due to the great dilution, although we are reminded, in this connection, of the failure of Basinger to influence the condition of cretinism in rabbits by repeated intravenous injection of serum from normal rabbits. If the serum was taken from rabbits who were being fed dried thyroid, the cretins improved, as by thyroid feeding. Hence when sufficient thyroid is fed to rabbits the presence of the thyroid hormone in systemic blood can be detected by the cretin test.

The influence of massage of the thyroid gland on the concentration of thyroglobulin in the thyroid lymph and in the neck lymph. In six experiments there was a slight increase in the concentration of thyroglobulin in the common neck lymph collected during 30 to 60 minutes' continuous massage of the thyroid gland. In three experiments no increase could be demonstrated. In no case where we were able to collect the lymph directly from the thyroid gland did we note increase in the concentration of thyroglobulin following massage of the gland. Massage of the thyroid increases the total output of lymph from the gland, therefore thyroid massage will increase the relative percentage of thyroid lymph in the

neck lymphatics. The increase of thyroglobulin in the neck lymphs following thyroid massage does not therefore necessarily mean an increase in the thyroid secretion rate. It may be simply an instance of more rapid elimination from the gland of a preformed secretion. It is obvious, however, that the massage will temporarily increase the quantity of thyroglobulin entering the venous blood by way of the neck lymph. But this fact does not permit us to conclude that the thyroid secretion rate has been augmented by massage.

We can offer no explanation of the negative results. In general, passive massage increases the blood flow through the gland by dilatation of the capillaries. We do not know how the rate of lymph production in the thyroids is related to the blood flow and blood pressure. It would seem that because of the increased output of lymph, massage of the gland should ultimately lead to a decreased concentration of thyroglobulin in the lymph, unless the massage at the same time increases the secretion rate. But in our experiments so far there has been no decrease in the thyroglobulin in the lymph after one hour's massage.

Influence of stimulation of the cervical sympathetic nerves on the concentration of thyroglobulin in thyroid lymph and neck lymph. In one experiment there was a slight increase of thyroglobulin in the common neck lymph collected during 30 minutes' intermittent stimulation of the cervical sympathetic nerves. In a second experiment, 60 minutes' stimulation of the nerves also gave a slightly positive result. Three experiments were distinctly negative, and in no case did we note any increase in thyroglobulin in the pure thyroid lymph during or following the stimulation of the cervical sympathetic nerves. It is difficult to interpret the two cases of slightly positive results because of the vasomotor changes in the head and neck region induced by the cervical sympathetic stimulation, and it is probable that these vasomotor changes induce changes in lymph production and lymph flow.

The influence of adrenalin and pilocarpin on the concentration of thyroglobulin in the thyroid lymph and the neck lymph. In two cases after pilocarpin, and in two cases after adrenalin, some increase in the concentration of thyroglobulin in the common neck lymph was found. Two experiments with pilocarpin and five experiments with adrenalin were negative. In no case did we note an increase of thyroglobulin in pure thyroid lymph after the intravenous administration of these drugs. The positive and negative findings were not related to the histological structure of the thyroids in the different animals.

Because of the inconstant results of these experimental procedures on thyroglobulin concentration in the common neck lymph, and the difficulty of interpretating the positive results, when present, we are now directing our attention to the thyroid vein blood. In dogs, at least, most

of the thyroglobulin passes directly into the blood. This is true even when the concentration of the hormone appears less in the blood of the thyroid vein than in pure thyroid lymph, because of the greater volume of blood going through the gland, as compared with the lymph output. Thyroid vein blood can also be obtained with less injury to the gland than can pure thyroid lymph. Moreover, because of the large volume of tissue lymph and slow flow of the lymph out of the gland, we do not know the factor of lag, while in the case of the venous blood the concentration of the thyroid hormone at any given moment must represent the thyroid activity one or two minutes prior to collecting the blood sample.

BIBLIOGRAPHY

(1) Basinger: Arch. Int. Med., 1916, xvii, 260.
(2) Cannon and Cattell: This Journal, 1916, xli, 58.
(3) Cannon and Smith: This Journal, 1922, lx, 476.
(4) Hektoen and Schulhof: Journ. Amer. Med. Assoc., 1923, lxxx, 386.
(5) Hektoen, Carlson and Schulhof: Journ. Amer. Med. Assoc., 1923, lxxxi, 86.
(6) Rogoff: Journ. Pharm. Exper. Therap., 1918, xii, 193.
(7) Van Dyke: This Journal, 1921, lvi, 168.
(8) Willier: Amer. Journ. Anat., 1924, xxx, 67.
(9) Csillag: Pflüger's Arch., 1924, ccii, 588.

THE PURIFICATION OF HEPARIN AND ITS PRESENCE IN BLOOD

W. H. HOWELL

From the School of Hygiene and Public Health, Johns Hopkins University

Received for publication November 28, 1924

In 1918 in a paper by Howell and Holt (1) a substance was described under the name of heparin which has a marked effect in preventing the coagulation of blood. The nature of its effect upon the processes of coagulation was investigated in some detail. Subsequently in a brief communication to the American Physiological Society (2) I described a second method of obtaining this material which gave a more constant and reliable product in a form suitable for laboratory experimentation.[1] On account of the high phosphorus content (about 5 per cent) of the product obtained it was assumed that the substance might belong to the group of phosphatids, but more recent work has shown that this assumption was incorrect. The active material contains no phosphorus. The material as obtained by the second method is water soluble, free from protein and will prevent coagulation completely in vitro and in vivo in concentrations of 1 mgm. or less to 5 cc. of blood. When injected intravenously in concentrations sufficient to render the blood completely incoagulable it has no apparent injurious effects, and repeated injections at intervals of some days cause no reactions of a toxic character. Experience has shown that the material obtained by either of the methods referred to is not a pure product. It contains an active element mixed with inert materials of various kinds. I have attempted to isolate the active substance by a number of different methods which have been more or less satisfactory. The most successful of these has been the use of cadmium chloride to remove the accompanying impurities. The method is as follows. The crude heparin prepared from dried dog's liver, according to the method described in 1922, is dissolved in water and digested at 38°C. for several hours with Taka-diastase or filtered saliva to remove a certain amount of glycogen which is usually present. The solution is then evaporated to dryness, the residue is powdered and is boiled for two hours in methyl alcohol, 95 per cent, in order to make insoluble any protein material added in the diastase treatment. After pouring off the alcohol and drying the

[1] The product as prepared by this method may be obtained from Hynson, Westcott and Dunning, Baltimore, Md.

residue on the water bath it is extracted with water and the extract is centrifugalized to get rid of insoluble material. The clear extract is then precipitated by the addition of an equal amount of acetone. This precipitate is centrifugalized off, dissolved in water and treated with a 10 per cent solution of cadmium chloride until no further precipitation occurs. The solution is centrifugalized and the supernatant liquid is treated with ammonia and sulphuretted hydrogen to remove the cadmium. The cadmium sulphide is removed by centrifugalizing or filtering and the liquid is evaporated to dryness on the water bath. The residue thus obtained is extracted with water and the turbid solution is filtered by suction through washed "filter cell" in a Buchner funnel. The filtrate is again evaporated to dryness and then extracted with a 0.5 per cent solution of sodium chloride. There is some insoluble residue in this extraction and the solution shows some opalescence. A process of extraction with water, filtration and evaporation to dryness must be repeated a number of times until the aqueous extract is entirely clear and leaves no residue. The final solution, which contains some sodium chloride owing to its use in the first extraction, is precipitated by an equal volume of acetone. The precipitate is centrifugalized off, is dissolved in 0.5 per cent solution of sodium chloride (some salt must be present for acetone precipitation) and again precipitated by an equal volume of acetone. This precipitate is again centrifugalized off, the supernatant liquid is decanted, and to remove the trace of sodium chloride in the precipitate, a mixture of equal parts of acetone and water is layered over it and allowed to stand for an hour or more. The use of dialysis to remove the salt is not advisable since the active substance dialyzes slowly through collodion and there would be loss of material. The precipitate is finally dissolved in water, filtered and evaporated to dryness. The material is obtained in the form of slightly colored scales which are readily soluble in water. The yield obtained by this method of purification is small, about 18 to 20 mgm. from 1 gram of crude heparin, since some of the active material goes down with the cadmium precipitate probably by adsorption. It shows however a greatly intensified activity. One milligram will prevent completely the coagulation of 40 cc. or more of cat's blood, an anticoagulant action far in excess of that shown by hirudin. Since the active element is precipitated by barium salts a further purification can be obtained by the use of barium chloride. This salt in 10 per cent solution is added until precipitation is complete, the precipitate after washing with cold water is dissolved in warm water and the barium is removed by the addition of dilute sulphuric acid in slight excess. The barium sulphate is removed by centrifugalizing, and the supernatant acid liquid, after the addition of sodium chloride to 0.5 per cent, is precipitated by an equal volume of acetone. The precipitate is sedimented by centrifugalizing, washed by layering over it a mixture of equal parts

of acetone and water, and is then dissolved in water, filtered and evaporated to dryness. The yield is small and in the form of nearly colorless scales, which show a high degree of anticoagulant activity. Owing to the small amount of material that has been available a thorough chemical examination has not been possible. The following positive and negative reactions have been noted.

1. All the protein reactions are entirely negative.

2. Reactions for sulphur and phosphorus are negative. The large phosphorus content of the crude material is obviously due to an impurity, which is removed with difficulty.

3. It is not precipitated by acids, nor by phosphotungstic acid in 5 per cent sulphuric acid.

4. It is not precipitated by salts of most of the heavy metals (Cu, Zu, Fe), but is precipitated by the sub-acetate of lead. I have used the absence of a precipitate on the addition of copper sulphate as an index of the purity of the preparation.

5. It is precipitated by barium chloride added in excess, the precipitate going into solution on warming and reappearing on cooling.

6. It contains nitrogen. A Kjeldahl determination (Arnold, Gunning and Dyer modification) on a specimen purified by the cadmium method and freed from salts by dialysis for several hours in a collodion sac, gave 2.7 per cent N.

7. It gives a Molisch reaction indicating the presence of a carbohydrate grouping.

The chemical nature of heparin remains undetermined, but it is evidently a stable substance that stands treatment by the methods of chemical analysis and purification. When it is obtained in larger amounts it should be possible to establish its chemical structure. Three of the reactions that it exhibits may be used for its identification: 1, its strong anticoagulant action; 2, its rather characteristic reaction with barium salts; 3, its ability to cause the formation of blood antithrombin when added to plasma or serum, a reaction referred to in the following paragraph.

The reactions of heparin in blood with the factors of coagulation, described in the original paper with Holt, have been verified for the purified product. The most significant results are the following: 1. Heparin, unlike hirudin, does not prevent the action of thrombin on fibrinogen. Mixtures of purified thrombin and heparin, even when incubated together for hours, cause prompt clotting when added to a solution of fibrinogen. 2. Heparin when added to plasma or serum causes the formation of an additional amount of antithrombin. If the plasma or serum is first heated to 70°C. this reaction does not occur. There is, therefore, in blood a thermolabile substance which reacts with heparin to form blood antithrombin. The nature of this thermolabile constituent has not been determined.

Its thermolability and its precipitation by dilute acids and by ammonium sulphate at concentrations of from 30 to 50 per cent make it probable that it is a protein of the nature of or associated with the euglobulin. Direct experiments indicate that it is not prothrombin inasmuch as solutions may be prepared containing prothrombin which do not react with heparin to form antithrombin.

Since heparin itself is not an antithrombin the question arises as to how it acts in preventing coagulation of the blood. Several possibilities have been considered. First, that it may act by influencing the condition of the calcium in the blood, for example, by combining with it to form an undissociable compound thereby reducing the concentration of ionized calcium below the limit necessary for the activation of prothrombin to thrombin. This explanation is made improbable by the fact that addition of calcium chloride in large excess to a mixture of blood and heparin does not induce coagulation. Second, the fact that heparin when added to blood causes the formation of large amounts of a true antithrombin suggests the possibility that the heparin may be changed to antithrombin or be combined in some way to form antithrombin and thus prevent coagulation. This view is made somewhat improbable by the consideration that heparin is a very stable compound while antithrombin is relatively unstable. Heparin, for example, is entirely thermostable at 100°C., antithrombin has its characteristic action destroyed at 70°C. Heparin may be precipitated uninjured by acetone and other precipitants and be carried through a long procedure of purification, while antithrombin is destroyed, so far as its action in coagulation is concerned, by precipitation with acetone and other reagents. But it is possible that heparin in combination may react differently from the uncombined material. The hypothesis was therefore submitted to an experimental test based upon the relation between antithrombin and metathrombin. In a series of papers from my laboratory by Weymouth (3), Gasser (4) and Rich (5) it has been shown that when thrombin and antithrombin are together in solution metathrombin is formed, and the presence of the latter may be demonstrated by reactivating it to thrombin by the alkali method as used by Morawitz. If, therefore, heparin is added to oxalated plasma, and then sufficient calcium to neutralize the oxalate and activate the prothrombin, we should assume that if the heparin has been changed to antithrombin and the calcium activates the prothrombin to thrombin there ought to be on standing a formation of metathrombin. The following experiment was made. Oxalated plasma was obtained from cat's blood. A portion of this was heated to 56°C. to remove the fibrinogen, which was centrifugalized off. The heated plasma was divided into two portions. To one there was added enough calcium chloride to provide for the activation of the prothrombin. To the other there was added first some heparin

and then the calcium chloride. Both preparations were put aside and examined for metathrombin at the end of 24 hours and 48 hours. In making this examination the solution was first heated to 60°C. to destroy the thrombin and prothrombin, and then an equal volume of N/10 NaOH was added and the preparation allowed to stand for 10 to 12 minutes. It was then neutralized by N/10 HCl and the presence of thrombin was tested for by adding some of the solution to an equal volume of freshly prepared fibrinogen. It was found that the plasma to which calcium alone was added contained metathrombin, while the one containing heparin and calcium had no metathrombin. Presumably therefore in this latter solution the heparin was not changed to antithrombin. A comparison experiment which gave an interesting result was made with serum obtained by adding enough calcium chloride to the oxalated plasma to cause clotting. This serum was divided into two portions, one with and the other without heparin. They were allowed to stand and were examined for metathrombin after 24 and 48 hours. It was found that the serum developed metathrombin, but that the serum plus heparin contained no trace of metathrombin. It would seem, therefore, that heparin not only is not changed over to an antithrombin, but in solutions (serum) containing both thrombin and antithrombin the production of metathrombin is prevented by the presence of the heparin.

The third possibility in regard to the action of the heparin is that it combines with or reacts with the prothrombin so as to prevent its activation to thrombin. This is the view that I have adopted in my previous publications. It is in accord with all the facts known, and by exclusion would seem to be the only explanation left. When prothrombin is isolated by the acetone method it is readily demonstrated that the presence of heparin will prevent its activation on the addition of calcium, but the solutions of prothrombin obtained by this method contain other constituents of the blood plasma, and therefore the experiment can not be accepted as direct proof of the action of heparin on prothrombin. To make the experiment decisive it will be necessary to devise a method for obtaining the prothrombin in pure condition. So far as our knowledge goes at present we are justified in believing that heparin prevents clotting of blood in its initial stage by a reaction with the prothrombin of the plasma which makes impossible its normal activation to thrombin.

Heparin is obtained from the liver (dog) and presumably is formed normally by some one of the tissues of this organ. There is some evidence also that it occurs in other tissues, e.g., the uterine mucous membrane (6) and the lymph glands (1). But in reference to its relations to coagulation it is especially important to determine whether it occurs in blood. In view of its great potency as an anticoagulant it is evident that if it occurs in normal blood it must exist in very small concentrations. In

incoagulable bloods, such as the blood of a peptonized dog, or in a slow clotting blood, such as that of hemophilia, one might expect to find it in greater amounts. Experiments have been made with normal blood plasma and with peptone plasma which gave satisfactory results.

Peptone plasma. A fasting dog weighing 8 kilograms was given an injection of 33 cc. of a 10 per cent solution of peptone made up by boiling in a 1 per cent solution of sodium chloride. The injection was made into the femoral vein under a local anesthetic, and after 20 minutes the animal was bled from the femoral artery through a paraffined cannula into paraffined centrifugal tubes. After centrifugalizing the clear plasma was pipetted off. It was entirely incoagulable. The plasma was precipitated by the addition of an equal volume of acetone, and the precipitate was centrifugalized and collected and then boiled for 2 hours in 95 per cent methyl alcohol, using a reflux condenser. The material was then dried and powdered and extracted for 15 minutes with a 1 per cent solution of sodium chloride. After filtration this solution was precipitated by an equal volume of acetone and the precipitate was collected by centrifugalizing and was dried. An aqueous extract was made and after filtration was dried over the water bath and kept in a desiccator for examination. This material was tested for anticoagulating action and for its power to cause the formation of antithrombin when added to blood-plasma, with the following results.

Anticoagulating action. An extract was made in 1 per cent solution of sodium chloride and filtered. Its action was tested upon cat's blood taken from the jugular vein with a syringe in comparison with a control of 1 per cent solution of sodium chloride.

1 cc. blood + 1.0 cc. extract = no clot in 24 hours
1 cc. blood + 0.5 cc. extract = partial clot in 3 hours
1 cc. blood + 1.0 cc. saline = clot in 15 to 20 minutes
1 cc. blood + 0.5 cc. saline = clot in 15 to 20 minutes

This experiment demonstrated that anticoagulant material was present in the extract of the peptone plasma preparation, and in view of the procedure by which it was obtained this material could scarcely be anything other than heparin. This conclusion was further borne out by the following experiment showing its power to form antithrombin when added to plasma.

Antithrombin formation. Oxalated plasma was obtained from cat's blood in the usual way. Some of this was heated to 56°C. in a water bath to precipitate the fibrinogen. The heated plasma was divided into two portions. To portion I some of the preparation from the peptone plasma was added in the proportion of 10 drops of the former to 6 drops of the latter. To portion II some 1 per cent solution of sodium chloride was

added in the same proportion. After standing an hour the two solutions were tested for their content in antithrombin according to the method I have described. The tests were made with a freshly prepared solution of fibrinogen made from the oxalated plasma of cat's blood, and with a solution of thrombin in 1 per cent solution of sodium chloride, 10 mgm. to 10 cc. The strength of the thrombin was such as to give the following coagulation times for the fibrinogen used.

Thrombin 1 drop fibrinogen 8 drops clot in 9 to 10 minutes
Thrombin 2 drops fibrinogen 8 drops clot in 4 minutes
Thrombin 3 drops fibrinogen 8 drops clot in 3 minutes
Thrombin 4 drops fibrinogen 8 drops clot in 2 minutes

The results of the examination for antithrombin are shown in the following table.

THROMBIN	SOLUTION		INCUBATION PERIOD	FIBRINOGEN	TIME OF CLOTTING
drops		*drop*	*minutes*	*drops*	
1	I	1	15	8	No clot in 24 hours
1	II	1	15	8	Clot in 55 to 60 minutes
2	I	1	15	8	No clot in 24 hours
2	II	1	15	8	Clot in 15 minutes
3	I	1	15	8	No clot in 4 hours, clotted overnight
3	II	1	15	8	Clot in 10 minutes
4	I	1	15	8	No clot in 4 hours, clotted overnight
4	II	1	15	8	Clot in 8 minutes

It will be seen from these results that the preparation made from the peptone plasma when added to heated oxalated plasma caused the production of a relatively large amount of blood-antithrombin, and since this is a characteristic reaction of heparin it justifies the conclusion that the anticoagulant obtained from the peptone plasma was heparin. We may further conclude that when a peptone solution is injected into a dog intravenously it causes an output of heparin into the blood, thus explaining the incoagulability of so called peptone blood.

Normal plasma. For the normal plasma a fasting dog of about the same weight was bled through a paraffined cannula into centrifugal tubes containing the proper amount of potassium oxalate, 1 per cent, made up in 0.9 per cent solution of sodium chloride. The blood was centrifugalized and the clear plasma was pipetted off. This plasma was put through the same process as in the case of the peptone plasma. The final precipitate

obtained by the addition of an equal volume of acetone was very small. It was dried and then extracted with a 1 per cent solution of sodium chloride. The filtered extract was tested for the presence of oxalate by the addition of a few drops of a 0.5 per cent solution of calcium chloride but none was found. This solution was tested, as in the case of the peptone plasma, for its direct anticoagulant action, and for its power to increase the content of antithrombin when added to plasma. The following results were obtained.

Anticoagulant action. One cubic centimeter of cat's blood (taken from the jugular vein) plus 1 cc. of the extract clotted in 50 to 55 minutes. One cubic centimeter of cat's blood plus 1 cc. of a 1 per cent solution of sodium chloride clotted in 17 minutes. Evidently the extract contained a small amount of anticoagulant material.

Antithrombin formation. Oxalated blood-plasma obtained from a cat was heated to 56°C. to remove the fibrinogen and was filtered. With the filtered solution two preparations were made. Solution 1 contained 1 cc. of the heated plasma plus 1 cc. of the extract. (This mixture gave a precipitate of calcium oxalate owing to the calcium chloride that had been added to the extract.) Solution 2 contained 1 cc. of the heated plasma plus 1 cc. of a 1 per cent solution of sodium chloride.

After standing an hour the two solutions were examined for their content in antithrombin with the result shown in the following table.

THROMBIN	SOLUTION		INCUBATION PERIOD	FIBRINOGEN	CLOTTING TIME
drops		*drop*	*minutes*	*drops*	
2	1	1	15	10	1 hour 25 minutes
2	2	1	15	10	35 minutes
3	1	1	15	10	30 minutes
3	2	1	15	10	20 minutes
4	1	1	15	10	20 minutes
4	2	1	15	10	15 minutes

The difference in the amount of antithrombin is shown most clearly with the weakest concentration of thrombin, but is indicated in all the solutions. The anticoagulant material obtained from the normal blood plasma gives therefore the characteristic reaction for heparin.

I have attempted by the same method to demonstrate the presence of heparin in freshly prepared dog's serum. Under a local anesthetic the femoral artery was cannulated and the blood was received through a paraffined cannula into centrifugal tubes. After the blood was firmly clotted the tubes were centrifugalized and 160 cc. of clear serum were ob-

tained. This serum was put through the procedure described above, and the final acetone precipitate after drying was dissolved in 2 cc. of a 1 per cent solution of sodium chloride and filtered. The filtrate was tested for its anticoagulant action on cat's blood taken from the jugular vein with a syringe. The result was negative.

1 cc. extract plus 1 cc. cat's blood = clot in 18 minutes
1 cc. 1 per cent sodium chloride plus 1 cc. cat's blood = clot in 15 minutes

It would seem, therefore, that in the act of clotting the heparin present in the normal plasma is combined in such a way that it can not be extracted by the method used, or else it is held within the clot.

These experiments give direct support to the view that heparin is a normal constituent of blood plasma and that it is greatly increased in the dog by the process of peptonization. These two facts make it probable that this substance is the inhibiting factor responsible for maintaining the fluidity of the blood under normal conditions. Variations in its amounts in the blood would explain differences in coagulation time and especially the marked delay found in hemophilia. I hope later to test this view by an examination of hemophilic blood according to the method outlined above. According to the theory of coagulation that I have proposed in other papers (1), the inhibitory influence of the heparin is neutralized when blood is shed by the phosphatid material (cephalin) liberated from the tissue-cells or from the disintegrated corpuscles (platelets) of the blood itself.

SUMMARY

1. A method is described for purifying heparin which yields a product capable of preventing coagulation in concentrations of 1 mgm. to 40 cc. of blood.

2. Some of the chemical reactions of heparin are described. It is free from phosphorus but contains 2.7 per cent of nitrogen.

3. Heparin is not an antithrombin, that is, does not prevent the reaction between thrombin and fibrinogen, but when added to blood it causes the formation of antithrombin by a reaction with a thermolabile constituent of the plasma.

4. Heparin prevents coagulation by a reaction with prothrombin which interferes with the activation of the prothrombin to thrombin.

5. Evidence is given to show that heparin is present in the plasma of normal blood and that the amount is greatly increased in dogs by the injection of peptone. The incoagulability of peptone plasma is due to the increased production of heparin.

6. It is suggested that heparin constitutes the inhibitory factor which

preserves the fluidity of circulating blood. In shed blood its action is neutralized by the phosphatid material furnished by the corpuscles of the blood or the cells of the tissues.

BIBLIOGRAPHY

(1) HOWELL AND HOLT: This Journal, 1918, xlvii, 328.
(2) HOWELL: This Journal, 1922, lxiii, 434.
(3) WEYMOUTH: This Journal, 1913, xxxii, 266.
(4) GASSER: This Journal, 1917, xlii, 378.
(5) RICH: This Journal, 1917, xliii, 549.
(6) KING: This Journal, 1921, lvii, 444.

ON THE EFFECT OF CHLORAL HYDRATE ON THE HEART AND CROSS STRIATED MUSCLE

STEPHEN D'IRSAY AND WALTER S. PRIEST

From the Cardiographic Laboratory and the Otto Baer Fund for Clinical Research of the Michael Reese Hospital, Chicago

Received for publication December 1, 1924

The work with which the following report deals was undertaken in an attempt to establish a method whereby those physiological properties of the heart dependent on nervous elements could be separated from those dependent on muscular elements. With such a method at our disposal it was hoped that interesting data concerning the effect of certain drugs on the heart could be obtained.

Rhode (1), working with chloral hydrate, believed that it was possible to eliminate functionally the physiological properties of the heart dependent on nerve centers or ganglia. Carlson (2), working with chloral hydrate on the heart of Limulus, found paralysis of motor centers, nerve fibers and muscle to take place in the order named. There was an interval of from 10 to 30 minutes after the paralysis of the nerve centers in which the nerve fibers were active and the muscle still responded to stimuli. And after paralysis of the nerve fibers there was another interval in which the muscle responded to direct stimulation. In addition to the above observations it has been shown that chloral hydrate exerts a negative influence on the dromotropic (3), inotropic and bathmotropic (4) functions of the heart.

Accordingly chloral hydrate was selected as the agent most likely to produce the effects we desired to obtain.

As a preliminary study, the effect of the drug on simple nerve-muscle preparations was first determined. Frogs (Rana americana) were used throughout. After pithing the animal, the heart was exposed and a three-way cannula inserted into the left truncus arteriosus. One arm of the cannula was connected by rubber tubing to a bottle containing Ringer solution, a second arm to a bottle containing chloral hydrate in Ringer solution, while the third arm was connected to a short upright piece of glass tubing to be used as a manometer for gauging the pressure of the perfusing fluid at the heart.

Perfusion was started with Ringer solution and the heart observed until it was seen to distend with the solution returning by way of the venous

tree. This was considered to be evidence that the perfusing fluid was reaching the tissues upon which the experiment was to be performed. A hole was then cut in the auricles and ventricle to allow the returning fluid to escape.

The left sciatic nerve was then carefully dissected out, care being taken to injure the blood supply as little as possible, and a loop of thread passed around the nerve to facilitate placing it on the points of a stimulating electrode when desired. The tendon of insertion of the corresponding gastrocnemius muscle was attached to a compound recording lever so arranged that the shadow of its vertical arm would fall on the horizontal slit of the electrocardiographic camera. Movements of the lever were recorded photographically on the moving film in the camera.

While the preparation was still being perfused with Ringer solution, stimulation, first of the nerve, then of the muscle directly, was done and tracings taken of the resulting contractions. Thus each experiment had a control tracing. Single "make" induction shocks were used, the key being connected in circuit with a signal lever which moved in front of the electrocardiographic camera so that the time of stimulation was recorded on the film.

After securing the control tracing, the Ringer solution was shut off and the chloral hydrate solution allowed to run in at the same rate, as determined by the manometer arm of the cannula. Successive stimulations, first of the nerve and then of the muscle direct, were made at intervals of from five to ten minutes and the results recorded graphically as above.

The first experiments were made with a 10 per cent solution of chloral hydrate, but this strength of solution caused paralysis of both nerve and muscle so quickly that no conclusions could be drawn. Paralysis occurred within 5 to 10 minutes and practically simultaneously, that is, when the nerve became paralyzed the muscle did not respond to direct stimulation. This did serve to show, however, that the solution introduced into the truncus arteriosus was actually reaching the nerve-muscle preparation.

Successive experiments were then made with lower concentrations of chloral hydrate solution until, with a 0.5 to 0.25 per cent solution, results were obtained which were uniform and quite suggestive. Summarizing these results, we found that paralysis of the nerve occurred from 15 to 90 minutes after beginning of the perfusion with chloral hydrate. Paralysis of the muscle occurred 35 minutes to $4\frac{1}{2}$ hours later. The threshold of stimulation was raised, as shown by the increase in potential difference of current necessary to obtain contraction. The nerve failed to conduct a minimal stimulus 30 to 60 minutes before the muscle failed to respond to an equal stimulus when applied directly, that is, the raising of the

threshold was noted on the nerve in each instance some time before the muscle. The strength of contraction diminished progressively. The length of time required for paralysis of the nerve and muscle increased as the strength of the solution decreased but not in direct proportion. The latent period of direct muscle stimulation gradually increased until paralysis of the nerve occurred, but after this the latent period remained constant until the muscle was completely paralyzed. The length of time during which the muscle responded to direct stimulation after paralysis of the nerve, bore a constant relationship to the total duration of the experiment regardless of the strength of solution used. This was from 66⅔ to 70 per cent. Table 1 gives the results in five experiments in which concentrations of from 0.5 per cent to 0.25 per cent were used, also the corresponding measurements when the perfusing fluid throughout was Ringer solution.

TABLE 1

Results obtained in a series of nerve-muscle perfusion experiments showing effect of solutions of chloral hydrate on period of latency, height of contraction, length of time required for paralysis of nerve and muscle respectively, and percentage relationship

EXPERIMENT NUMBER	STRENGTH OF SOLUTION	PARALYSIS OF NERVE	PARALYSIS OF MUSCLE	HEIGHT OF CONTRACTION AT BEGINNING OF EXPERIMENT	HEIGHT OF CONTRACTION AT END OF EXPERIMENT	LATENT PERIOD. NERVE STIMULATION. BEGINNING OF EXPERIMENT	LATENT PERIOD. NERVE STIMULATION. END OF EXPERIMENT	LATENT PERIOD. DIRECT MUSCLE STIMULATION. BEGINNING OF EXPERIMENT	LATENT PERIOD. DIRECT MUSCLE STIMULATION. END OF EXPERIMENT	PERCENTAGE OF TOTAL DURATION OF EXPERIMENT IN WHICH MUSCLE ALONE RESPONDS TO STIMULATION
	per cent	*minutes*	*minutes*	*cm.*	*cm.*	*second*	*second*	*second*	*second*	
IV	0.5	15	50	10	1.5	0.04	0.08	0.06	0.12	70
V	0.5	15	50	3.7	0.3	0.02	0.04	0.04	0.12	70
VI	0.5	15	50	1.7	0.4	0.02	0.04	0.04	0.06	70
VII	0.25	90	280	1.0	0.1	0.04	0.08	0.06	0.16	68
VIII (control)	Ringer solution	None	None	4.8	4.8	0.02	0.02	0.04	0.04	
IX	0.25	55	165	3.1	0.7	0.02	0.12	0.04	0.12	66⅔

Thus the selective action of chloral hydrate in paralyzing first nervous then muscular tissue, as noted by Carlson in his Limulus experiments, was found to hold when applied to a nerve-muscle preparation of the frog.

The control experiments, that is, the ones in which Ringer solution alone was used as the perfusing fluid, could be continued almost indefi-

nitely without change in the latent period or threshold of stimulation, and while in some there was a variation in the height of contraction there was no definite, progressive diminution in the height of contraction as was the case in the chloral hydrate treated preparations.

Figure 1 is a graph illustrating the effect of perfusion with a 0.5 per cent solution. *A* represents the results obtained when the nerve was stimulated, the upper curve representing the height of contraction of the muscle,

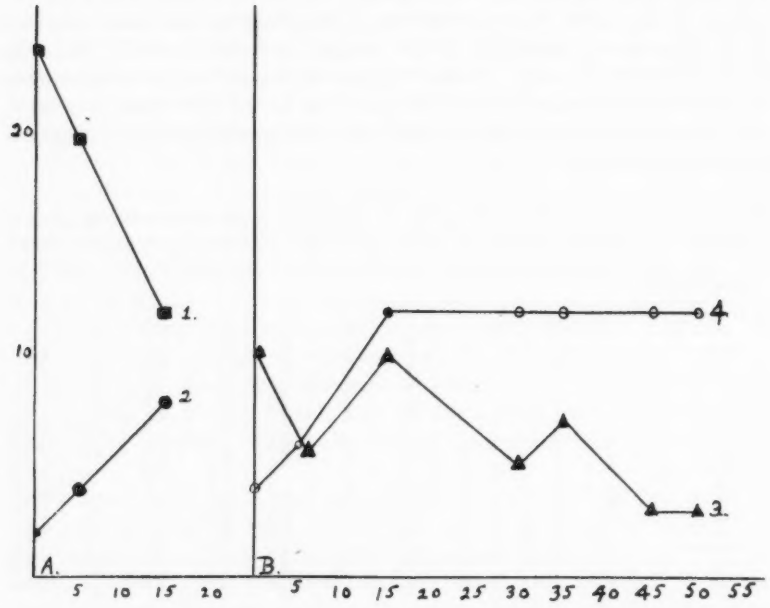


Fig. 1. Effect on height of contraction and latent period. Nerve-muscle preparation treated with 0.5 per cent chloral hydrate solution. *A*, Nerve stimulation. *1*, height of contraction. *2*, latent period. *B*, Direct muscle stimulation. *3*, height of contraction. *4*, latent period. Each division along abscissa = 0.01 second in considering the latent period and 1 mm. in considering the height of contraction. Duration of experiment in minutes plotted on ordinates.

the lower curve representing the latent period. Each division along the abscissa represents 0.1 second in considering the latent period and 1 mm. in considering the height of contraction. The ordinates represent time in minutes. *B* represents the results obtained on stimulation of the muscle directly, the upper line being the height of contraction, the lower the latent period. It is seen that the latent period does not increase after the nerve is paralyzed. Also that the length of time during which the

muscle still responds to direct stimulation after paralysis of the nerve represents 70 per cent of the total duration of the experiment.

For the recording of experiments on the heart, it was thought that simultaneous electrocardiograms and myograms of auricle and ventricle would furnish some of the data required. Accordingly the preparation was arranged as follows:

A small three-way cannula was inserted in the inferior vena cava as far removed from the heart as possible. One arm of the cannula was connected with a bottle containing Ringer solution, another to a bottle containing chloral hydrate solution and the third arm connected to a manometer tube. This arrangement, except for the vessel in which the cannula was tied, was similar to that used in the nerve-muscle experiments.

Suspended above the heart were two non-polarizable clay "boot" electrodes containing 10 per cent zinc sulphate solution and in each boot was a small strip of zinc having a binding post on the top. These two electrodes were connected to the galvanometer of the electrocardiograph. The feet of the "boot" electrodes were covered with a thick kaolin paste imbedded in which were pieces of moist wool yarn. The end of one piece of yarn was placed on the apex of the ventricle, and the end of the other on the right auricle as near the sinus as possible. By means of these non-polarizable electrodes, electrocardiograms of the heart could be obtained.

The ventricle was connected to a recording lever like that used in the nerve-muscle experiments, likewise the left auricle. These levers were made of very light material and the attachment to the heart tissue made by means of a very fine copper wire hook on the end of a thread. In making the attachment the heart was not handled in any way, the hooks simply being caught in the muscle. The whole recording device was arranged so as to cause as little interference as possible with the normal heart action. The vertical recording arm of each lever was so placed as to throw a shadow on the horizontal slit of the electrocardiographic camera. The precautions noted by Mines (5) in his perfusion experiments on the frog's heart were observed.

The electrocardiograms showed the usual auricular and ventricular deflections (fig. 3, *A*). These correspond to the movements of the capillary electrometer as used by Gotch (6) in his perfusion experiments on the frog's heart. *P* and *R* were uniformly upright. *T* was usually upright but showed considerable variation in height and configuration. Under the influence of chloral hydrate perfusion, however, there appeared a striking and practically constant change in this latter deflection. The amplitude of deflection was markedly increased, the upstroke began to rise immediately after completion of the downstroke of *R*, and the time required for the completion of the deflection was markedly prolonged (fig. 3, *B* and *C*).

Several experiments using normal Ringer solution as the perfusing fluid were first carried out and a range of normal for the following factors obtained; 1, Rate of heart beat. 2, P-R interval. 3, The interval between the beginning of the "P" wave of the electrocardiogram and the auricular myogram referred to hereafter as the P-R interval. 4, The interval between the beginning of the R wave of the electrocardiogram and the ventricular myogram, referred to as the "R-V" interval.

The normal values of the factors mentioned above were: rate 31 to 55 beats per minute; P-R, 0.24 to 0.36 second; P-A, 0.12 to 0.28 second; R-V, 0.12 to 0.28 second. The ventricular and auricular myograms varied more or less with each experiment, but as an initial tracing was made in each experiment while the preparation was still being perfused with Ringer solution, a normal was established in any given experiment for subsequent comparison. At times it was impossible to exclude from the ventricular myogram a slight deflection caused by movement of the auricle and vice versa, but this was not such as to interfere with analysis of the results.

Solutions of chloral hydrate of strengths found suitable for work on the nerve-muscle preparations were found to be too strong for work on the heart, paralysis occurring in from 15 to 20 minutes. Accordingly weaker concentrations were tried until, with solutions of from 0.0075 to 0.0025 per cent the following results were obtained. 1. There was a progressive slowing of rate, quite rapid at first then more gradual until complete paralysis of the sinus took place. This may be considered a negative chronotropic effect of the drug. 2. The P-R interval became much longer, that is, there was depression of conductivity or negative dromotropic effect. 3. The P-A and R-V intervals were lengthened. This effect is probably due to the influence of the drug on the latent period of the muscle, although the influence on irritability (negative bathmotropic effect) may also be a factor. 4. Depression of contractility is suggested by the change in the form of the myograms, especially of the ventricle, as perfusion with chloral hydrate progresses. The upstroke becomes more gradual and crawling in character (fig. 3, *B* and *C*). At the end of the experiment the auricular myogram becomes scarcely perceptible. 5. The possible effect of the chloral hydrate solution on muscular tonus could not be demonstrated definitely by the methods of our experiments. The change in position of the auricular and ventricular myograms with reference to the base line (fig. 3), does not in itself indicate tonus change, as it was necessary as the perfusion progressed to shift the stand on which the preparation was set up in order that the shadows of the myographs would constantly fall on the camera opening. The factor which made the shifting necessary was the gradual but progressive dropping from their original positions on the part of the myograph levers. This phe-

nomenon was not noted in the control experiments in which Ringer solution was the perfusing fluid. This we believe to be visible evidence, at least, of decreased muscle tone. The effect on the intervals mentioned in 2 and 3 reaches a maximum after a certain period of time and then the various intervals are constant for the remainder of the experiment.

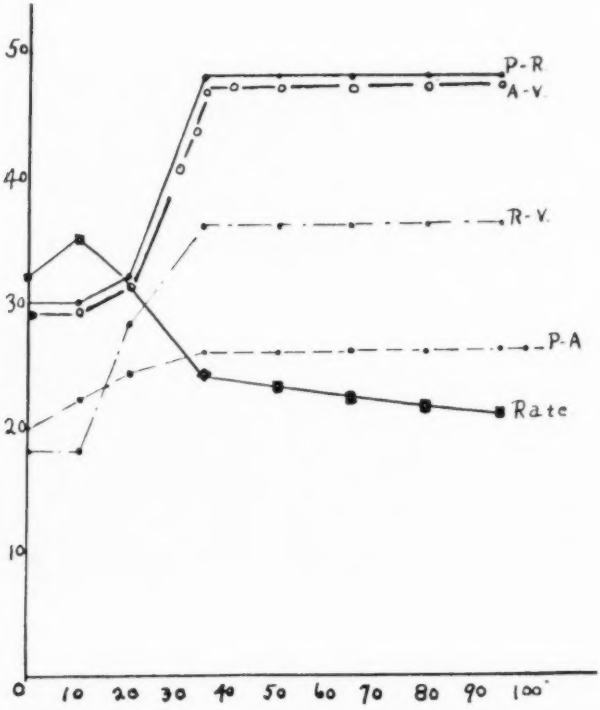


Fig. 2. Graph illustrating the effect on the frog's heart of perfusion with 0.0025 per cent chloral hydrate solution. Time in minutes plotted on ordinates. Each division on abscissa represents two beats per minute in considering the rate, and 0.02 second in considering the various intervals. *P-R*, interval between beginning of P wave and succeeding R wave of electrocardiogram. *A-V*, interval between deflection of auricular myogram and succeeding deflection of ventricular myogram. *R-V*, interval between R wave of electrocardiogram and corresponding ventricular myogram. *P-A*, interval between P wave of electrocardiogram and corresponding auricular myogram.

Figure 2 is a graph obtained by plotting the various intervals and rate against the time of the experiment. In this graph there is a curve, *A-V*, which represents the interval between the auricular and ventricular

myograms, but obviously this should correspond to the *P-R* interval. The strength of chloral hydrate solution used in this particular experiment was 0.0025 per cent. It will be seen that, with the exception of the rate curve, all the curves reach a level 35 minutes after the beginning of perfusion with chloral hydrate solution (zero on the time line). The rate shows an initial rise lasting 10 minutes, then an abrupt fall which gives way to a gradual fall after 35 minutes of perfusion. This gradual

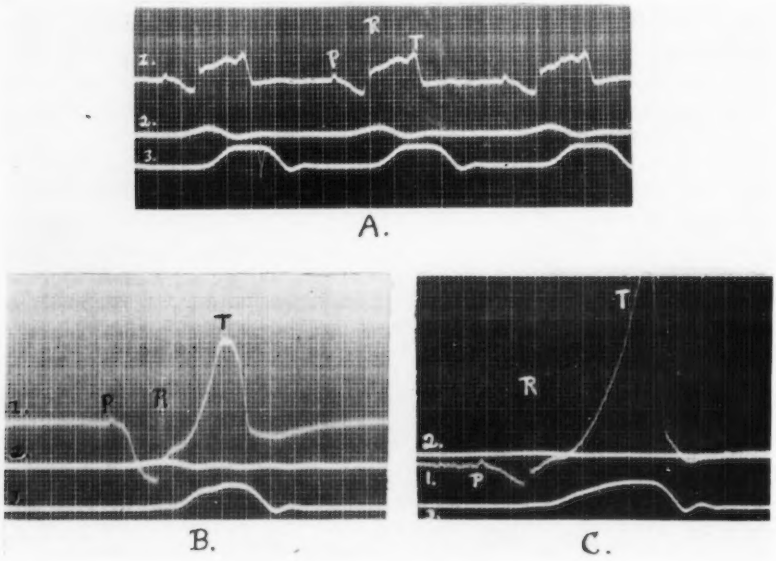


Fig. 3. Simultaneous electrocardiogram and auricular and ventricular myogram illustrating the effect of perfusion with 0.005 per cent chloral hydrate solution.

A. "Control" tracing, taken while perfusing with Ringer solution.

B. Sixty minutes after beginning of perfusion with 0.005 per cent chloral hydrate solution.

C. One hundred and seventy minutes after beginning of perfusion with 0.005 per cent chloral hydrate solution and just before paralysis of the sinus occurred. *1*. Electrocardiogram. *2*. Auricular myogram. 3. Ventricular myogram.

fall continues until paralysis of the sinus occurs. This 35-minute period represents 36.8 per cent of the total duration of the experiment. In ten other successfully completed experiments the period which elapsed from the beginning of perfusion with chloral hydrate until the curves reached a level and the rate curve changed from an abrupt to a gradual fall, bore the following percentage ratio to the total duration of the experiment: $33\frac{1}{3}$ per cent in six, 49 per cent in one, 25 per cent in one, 39 per cent in

one, 21 per cent in one. And this was regardless of the total duration of the experiment or the strength of solution used providing the concentration was between 0.0025 and 0.0075 per cent. This bears a close relationship to the time required for paralysis of the nerve in nerve-muscle preparations subjected to certain strengths of chloral hydrate solution.

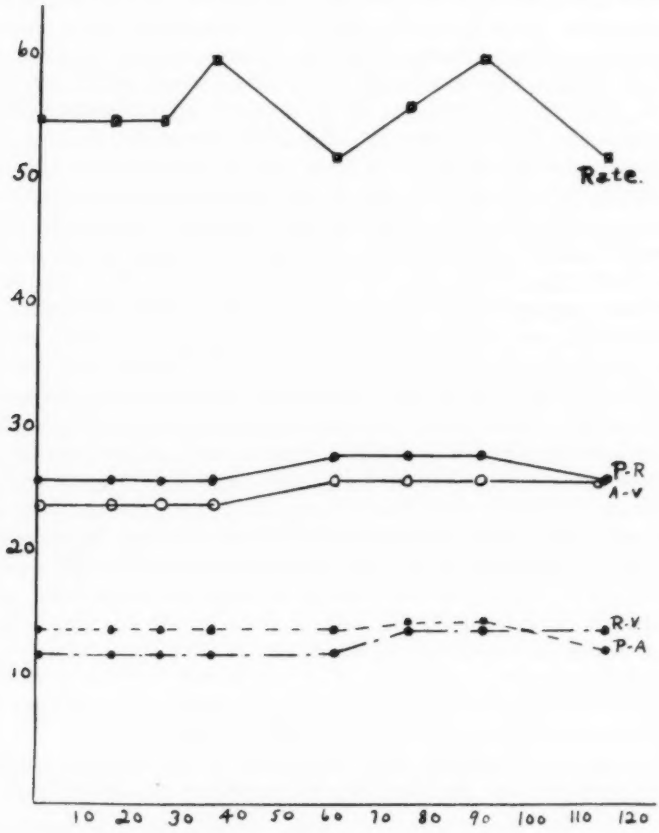


Fig. 4. Graph illustrating the effect on the frog's heart of perfusion with Ringer solution. Ordinates and abscissae represent same values as in figure 2. *P-R*, *A-V*, *R-V* and *P-A* denote corresponding intervals to those in figure 2.

Figure 3 shows three curves taken at different times during the course of a typical experiment. *A* shows the condition when Ringer solution was the perfusing fluid. *P-R* interval is 0.26 second, *P-A* is 0.16 second, *R-V* is 0.12 second, and the rate is approximately 42 per minute. *B* is

tracing made 65 minutes after perfusion with 0.005 per cent chloral hydrate solution was started. The *P-R* interval is 0.40, the *P-A* interval 0.24, the *R-V* interval 0.20 and the rate is 20 per minute (the cycle was too long to be included in the portion of the tracing reproduced here). It was at this point that the three intervals *P-R*, *P-A* and *R-V* reached a maximum and remained constant until the end of the experiment. *C* is from a tracing taken about 3 minutes before paralysis of the sinus and 170 minutes after beginning of perfusion with chloral hydrate. Here the *P-R* is still 0.40 second, the *P-A* 0.24 second, and the *R-V* 0.20 second. The rate has dropped to approximately 9 per minute. In this experiment the time required for the three intervals, *P-R*, *P-A* and *R-V*, to reach a maximum is 34.2 per cent of the total duration of the experiment. Figure 4 is a graph illustrating results obtained when Ringer solution alone was used as the perfusing fluid. The entirely different character of the curves as compared with figure 2 will be readily seen. Perfusion with Ringer solution could be continued for 4 or 5 hours without any appreciable alteration in the various intervals or rate.

SUMMARY AND CONCLUSIONS

1. Perfusion experiments were conducted on nerve-muscle preparations of frogs using various concentrations of chloral hydrate in Ringer solution.

With concentrations of from 0.25 to 0.50 per cent paralysis of the nerve occurred an appreciable length of time before the muscle failed to respond to direct stimulation. The length of time required for paralysis of the nerve represented from 30 to $33\frac{1}{3}$ per cent of the total duration of the experiment. During this time the period of latency gradually increased. Following paralysis of the nerve the latent period increased very slightly if at all. The threshold of stimulation of the nerve was rapidly raised, that of the muscle less so. The strength of contraction of the muscle gradually decreased.

None of the above effects were noted in control experiments in which Ringer solution alone was used as the perfusing fluid.

2. Perfusion experiments were conducted on the frog's heart in situ using various concentrations of chloral hydrate in Ringer solution.

Simultaneous electrocardiograms and auricular and ventricular myograms were made.

With concentrations of from 0.0075 to 0.0025 per cent gradual paralysis of the sinus occurred. This process was rapid at first then more gradual until the heart stopped in diastole. During this time there was a lengthening of the P-R interval (depressed conductivity), and lengthening of the intervals between the P and R waves of the electrocardiogram and the corresponding auricular and ventricular myograms (increased latent period). There was also a decrease in the rapidity of contraction of the ventricle.

The decrease in conductivity and increase in latent period reached a maximum at a point representing, on an average, 30 to 33⅓ per cent of the total duration of the experiment.

Reasoning from analogy with the results obtained on nerve-muscle preparations, we feel justified in concluding that this initial period represents the time during which both nervous and muscular elements of the conduction and myocardial systems are functioning. That paralysis of the nervous elements occurs coincident with the reaching of a level of certain factors mentioned above and that from then on the muscular elements alone function.

If this be true, it constitutes additional support to the theory that the various fundamental functions of heart tissue are under both myogenous and neurogenous control. The ratio between the two influences seems to vary according to the function analyzed. In the case of conductivity and latent period there seems to be a definite neurogenous element superimposed upon and easily distinguished from the myogenous element, whereas discrimination between the two is more difficult in the case of rate and tone. No definite data could be obtained on the function of irritability and very little on contractility. Every one of the fundamental functions, however, seems to be in operation even in the absence of nervous elements, but on a lower general level.

Inasmuch as, with proper concentrations of chloral hydrate solution, it appears possible to obtain quite a long interval (from one to two hours in favorable cases) in which the muscular elements alone are functioning, we believe that the use of this method of eliminating the nervous elements of cardiac mechanism might yield data as to the exact point of action of various drugs on the heart.

The authors are indebted to Prof. A. J. Carlson for his many helpful suggestions and criticisms of this work.

BIBLIOGRAPHY

(1) Rhode: Arch. f. exper. Path. u. Pharm., 1905, liv, 104.
(2) Carlson: This Journal, 1904, xii, 471; 1905, xiii, 217. Also a series of articles in 1906, xvi, and 1906–7, xvii.
(3) Harnack: Englemann's Arch., 1904, 415.
(4) Böhme: Arch. f. exper. Path. u. Pharm., 1905, lii, 346.
(5) Mines: Journ. Physiol., 1913, xlvi, 188.
(6) Gotch: Proc. Roy. Soc. Lon., 1907, lxxix B, p. 323.

THE ANTAGONISTIC ACTION OF CERTAIN SUGARS, AMINO ACIDS AND ALCOHOLS ON INSULIN INTOXICATION[1]

CARL VOEGTLIN, EDITH R. DUNN AND J. W. THOMPSON

From the Division of Pharmacology, Hygienic Laboratory, U. S. Public Health Service

Received for publication December 2, 1924

The object of this paper is to record some observations which, we believe, may assist in the elucidation of the still incompletely understood mechanism of the physiological action of insulin. The work is based on the well-known discovery of Banting, Best, Collip, Macleod and Noble (1) who found that the characteristic symptoms supervening in rabbits after the administration of an excessive dose of insulin can be rapidly relieved by the injection of glucose. The question naturally arises as to whether carbohydrates other than glucose, amino acids, alcohols and, generally, substances which may be converted within the body into glucose, have an antagonistic effect similar to that of glucose. This idea occurred also to Noble and Macleod (7) who put it to an experimental test. Their method is briefly the following:

After taking blood samples of normal rabbits the animals receive an injection of insulin in such amounts as to produce convulsions. As soon as the convulsions supervene, the substance to be tested for its antagonistic effect is injected subcutaneously and at intervals other blood samples are taken, these being analyzed for sugar. The disappearance of the symptoms is taken as a criterion of the activity of the substances tested. Noble and Macleod found that "the only sugar which can definitely antidote the symptoms that accompany the hypoglycemia due to insulin is glucose." Mannose was found almost as efficient as glucose. Levulose, galactose and maltose cause only a slight improvement in the symptoms, in spite of a marked increase in blood sugar. The rise in blood sugar in this case is evidently not due to glucose, but must be attributed to the presence of these reducing sugars. Arabinose, xylose, sucrose, lactose, sodium lactate and glycerol are inactive. From this it is evident that only two aldohexoses (glucose and mannose) are effective antagonists of insulin and that blood sugar estimations are only of limited value in the study of this kind of antagonism.

[1] In this paper the term "insulin intoxication" is used to denote the pathological condition produced by the administration of toxic doses of insulin to normal animals.

It is obvious that the method employed by Noble and Macleod is only adapted for the investigation of the antagonistic action of substances which are very easily and rapidly converted into glucose by hydrolysis, tautomeric change or other means. For this reason a method of broader scope would decidedly enlarge the usefulness of insulin in the study of metabolism. We have utilized for this purpose a method which we suggested recently for the biological standardization of insulin (10).

METHOD. Healthy, non-pregnant albino rats are kept on a standard diet for several weeks previous to the experiment. About 18 hours before the experiment the animals are placed in a ventilated incubator room (28° to 30°C.) and starved. The minimum lethal dose of insulin (defined as the lowest dose which yields a mortality of 90 to 100 per cent in a set of ten animals) is then determined by injecting the drug subcutaneously in graded doses, i.e., 1 cc., 2 cc., 4 cc., etc., per kilogram bodyweight. Having thus established the M.L.D. of insulin, we can proceed to test the antagonistic action of various substances. This is done by injecting several sets of 10 rats each, under the above mentioned conditions, with the M.L.D. of insulin, this being *immediately* followed by the administration, by means of a stomach tube, of graded doses of the substances to be tested. It was found that 8 grams of glucose per kilogram are required to counteract the fatal action of a M.L.D. of insulin. The following protocol illustrates such an experiment:

NUMBER OF RATS	INSULIN	GLUCOSE PER KILO	MORTALITY
	M.L.D.	*grams*	*per cent*
10 (controls)	1	None	100
10	1	5	20
10	1	6	10
10	1	8	0
10	1	10	0

In the same manner there was tested a considerable variety of other substances. It is of course always necessary to establish the toxicity of these substances before their antagonistic action against insulin is tested. Our method is limited to substances which have no considerable inherent toxicity, but fails for instance with acetic aldehyd, which was found to be quite toxic in the amounts required for our purpose.

The method has an accuracy of about 20 to 50 per cent or, in other words, it is possible to establish the protective dose of a given substance against insulin within 20 to 50 per cent. If the test be repeated several times it is quite feasible to reduce the error to 20 per cent. Some of the active substances were also tested as to their effect on the removal of the symptoms of insulin intoxication, thus making use of the method of Noble and Macleod.

In some cases blood sugar estimations were made on animals which had received a M.L.D. of insulin together with a protective dose of an active substance, in order to determine whether the blood sugar values remained normal. For this purpose the blood from several decapitated rats killed from 1 to 24 hours after injection was analyzed separately for blood sugar. Each figure in tables 4 and 5 therefore indicates the blood sugar of an individual rat.

DISCUSSION OF RESULTS. It has been pointed out that the method used by us yields information as to whether a certain substance given in proper amounts prevents the fatal action of a minimum lethal dose of insulin. We know from the work of the Toronto School that *glucose* may be considered as a physiological antidote of insulin and our work with standardized rats has shown that about 8 grams of glucose per kilo administered by mouth at the time of the subcutaneous injection of a M.L.D. of insulin will protect these animals from death. A larger dose of glucose is needed to completely prevent the hypoglycemia.

It will be seen from the data of table 1 that two other hexoses besides glucose, i.e., *galactose* and *fructose*, offer very good protection. These observations are somewhat at variance from a quantitative point of view with the *curative action* of these sugars as observed in rabbits by Noble and Macleod (7) and quite recently in mice by Herring, Irvine and Macleod (4). These investigators arrive at the conclusion that fructose and galactose are less efficient in alleviating the symptoms.

The four disaccharides tested, i.e., *maltose*, *lactose*, *sucrose* and *trehalose*, also exert a powerful preventive action, about equal to that of glucose.

Inulin yielded negative results, as would be expected. Hydrolysis of inulin yields fructose. The negative results obtained with this polysaccharide therefore indicate lack of digestion, and this view was confirmed by the necropsy, which showed the presence of large amounts of the undigested carbohydrate in the intestines.

Of the two alcohols included in this work, *glycerol* showed a remarkable effectiveness, whereas *mannitol* was only slightly active (table 2).

The antagonistic effect of glycerol on the hypoglycemia following insulin is illustrated by the data in table 4. It will be noted that the 12 control animals showed the usual gradual drop in blood sugar from an average value of 0.113 per cent before injection to 0.04 per cent after injection, whereas the animals having received 6 grams of glycerol per kilogram had a consistently higher blood sugar, and very mild symptoms. A larger amount of glycerol would undoubtedly have prevented the hypoglycemia.

In view of this very great *preventive* action of glycerol it was decided to carry out a few experiments on the *curative* action of the substance on the removal of already established symptoms. Observing the usual technic a minimum lethal dose of insulin was given to six rats. (Twelve con-

trols similarly injected all died within four hours.) The six animals received intraperitoneally from 1 to 6 grams of glycerol (20 per cent solution) per kilogram bodyweight. In every case there was noted a marked improvement in the severity of the symptoms, the convulsions or coma

TABLE 1

Sugars

		DOSE PER KILO	MORTALITY	NUMBER OF ANIMALS	MORTALITY OF CONTROLS RECEIVING ONLY INSULIN*
		grams	*per cent*		*per cent*
Pentoses	l-Arabinose	7	60	10	100
	Xylose	7	70	10	80
Hexoses	Glucose	6	30	10	100
		8	0	10	100
		10	0	10	100
	Galactose	7	20	10	80–100
		8	20	10	80–100
		10	0	10	80–100
	Fructose	3	45	20	80
		4	25	20	80
		5	17	30	80
		6	0	10	80
Disaccharides	Maltose	3	30	10	90
		4	20	10	90
		5	0	10	90
	Lactose	3	20	20	80
		4	20	20	80
		5	3	30	80
	Sucrose	3	37	30	80–90
		4	15	20	80–90
		5	10	20	80–90
		6	0	20	80–90
	Trehalose	6	0	10	80
Polysaccharide—Inulin		5	100	10	90
		6	90	10	90

* It will be noted that some of the control experiments yielded a slightly lower mortality than the definition of the M.L.D. for insulin requires. Under these circumstances the protective dose of the substances studied may be slightly lower than would have been the case had a full M.L.D. of insulin been given.

disappearing within ten minutes. Relapse occurred in two animals, but four out of the six survived.

TABLE 2

Alcohols, acids and amino acids

	DOSE PER KILO	NUMBER OF ANIMALS	MORTALITY	MORTALITY OF CONTROLS RECEIVING INSULIN ALONE
	grams		*per cent*	*per cent*
Mannitol	3	10	70	100
	4	10	30	100
	5	10	80	100
Glycerol	4	10	80	88
	5	10	0	88
	6	40	0	88
Lactic acid (sodium salt)	2	10	100	90
	4	10	90	90
Pyruvic acid* (sodium salt)	10.6	9	100	80
Glycin	6	10	100	80
	7	10	80	80
d-Alanin	6	10	20	70
	7	20	0	70
Glutaminic acid	2	20	95	100
	4	20	95	100

* Toxicity not determined on account of insufficient supply of substance.

TABLE 3

Insulin preceded by olive oil 4 to 6 hours

OLIVE OIL PER KILO	NUMBER OF ANIMALS	MORTALITY	MORTALITY OF CONTROLS RECEIVING ONLY INSULIN	INTERVAL BETWEEN OLIVE OIL AND INSULIN INJECTION
grams		*per cent*	*per cent*	*hours*
4.5	10	60	100	6
9	10	70	100	6
13.5	20	60	100	6
18	30	57	100	4–6
27	30	56	100	4–6
36	20	30	95	4

Whether glycerol is a direct antidote for insulin or whether it must be first converted into glucose cannot be decided on the basis of this evidence.

The latter possibility would presuppose a remarkable speed in transformation of glycerol into glucose.

Noble and Macleod failed to obtain any alleviation of the symptoms in rabbits as a result of subcutaneous injections of undiluted glycerol. The reason for the discrepancy between our observations and those of these workers may be due to differences in the mode of administration or

TABLE 4

Influence of glycerol on the hypoglycemia following insulin

INSULIN	GLYCEROL PER KILO	NUMBER OF ANIMALS	BLOOD SUGAR AFTER INJECTION				
			1 hour	2 hours	3 hours	4 hours	24 hours
	grams		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1 M.L.D.	6	12	0.116	0.068	0.083	0.095	0.115
			0.060	0.065	0.068	0.085	0.115
							0.113
							0.120
1 M.L.D. (controls)	None	12*	0.059	0.047	0.040		

* The other 9 animals died of insulin intoxication within 4 hours after injection.

TABLE 5

Influence of alanin on the hypoglycemia following insulin

INSULIN	ALANIN PER KILO	NUMBER OF ANIMALS	BLOOD SUGAR AFTER INJECTION				
			1 hour	2 hours	3 hours	4 hours	24 hours
	grams		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1 M.L.D.	7	12	0.067	0.068	0.066	0.057	0.170
			0.069	0.061	0.050	0.061	0.161
			0.082	0.065			
Average			0.073	0.065	0.058	0.059	0.165
1 M.L.D. (controls)	None	12*	0.063	0.054			

* The remaining 10 animals died of insulin intoxication within 3 hours after injection.

perhaps to a difference in the animal species.[2] In all events our observations agree with the results of Lüthje (6) who showed that glycerol can give rise to glucose and they harmonize also with some recent observations of Thomas (9) indicating that glycerol has a marked antiketogenic action in diabetes. Our results are also in agreement with the findings of

[2] Recently we were able to demonstrate the curative action of glycerol (2 to 6 gm. per kilo) administered intraperitoneally to rabbits in a 20 per cent solution.

Krogh and Lindhard (5) who showed that carbohydrate is formed from fat in man, when the respiratory quotient is below 0.8.

Lactic acid and *pyruvic acid* are supposed to be readily convertible into glucose in the mammalian body. These two acids were therefore tested in the form of the sodium salts as to their activity against insulin, with negative results. Noble and Macleod had previously found lactic acid inactive as a curative agent. The negative results with lactic acid are rather astonishing and indicate that, at least under the conditions of our experiments, the transformation of lactic acid into glucose does not take place at sufficient speed to prevent death from insulin. This absence of activity is not due to lack of absorption of the substance from the gastro-intestinal tract, as shown by the fact that this tract was found to be practically empty at necropsy.

The three amino acids—*glycin*, *d-alanin* and *glutaminic acid*—showed that alanin is remarkably active in preventing insulin death, being about as effective as glucose.

Glycin gave negative results which may be due to a slow rate of absorption from the intestines. The latter, at necropsy, were found to be filled with a large amount of fluid, presumably containing the unabsorbed amino acid. Glutaminic acid also was inactive. The influence of alanin given simultaneously with insulin on the hypoglycemia following the latter is illustrated by the data in table 5. It will be noted that alanin decreases the hypoglycemic effect considerably and there is no doubt that a larger dose of the amino acid would prevent altogether the occurrence of hypoglycemia.

The results with these three amino acids are of interest in connection with the question of their conversion in the body into glucose. It is well known that Ringer and Lusk (8) have shown that alanin fed to a phloridzinized dog results in an "extra" elimination of glucose in the urine, the increase of glucose corresponding to 92 per cent of the theoretical amount, assuming complete conversion. These results were confirmed by Dakin and Dudley (3). Similar results with glycin, glutaminic acid and some other α-amino acids (in the phloridzinized dog) indicated conversion into glucose. Another method used consists in the determination as to whether or not the isolated liver on perfusion with the substance to be tested has the ability to form glucose. Both of these methods have their weak points, which, for instance, leads Dakin (2) to the belief "that a direct conversion of the carbon of glycin in glucose does not take place but rather that it causes a disturbance in the normal equilibrium existing between the amino acids or peptides in the body tissues with the result that other amino acids capable of furnishing glucose (e.g., alanin) are set free." In view of this uncertainty of the reliability of the above results, it is certainly gratifying to know that the evidence as far as alanin is concerned, seems to be con-

firmed by our experiments on the antagonistic action of alanin against insulin. We fully realize that the latter evidence is also of an indirect nature and we do not want to fail to mention that the antagonism between alanin and insulin may perhaps be due to the possibility that alanin by being metabolized has a sparing action on glucose. However, this interpretation probably is not correct in view of the fact that the amount of alanin necessary to antagonize a M.L.D. of insulin is practically the same as in the case of glucose.

Inasmuch as glycerol proved itself to be such a powerful physiological antagonist against insulin it was of interest to test out the effectiveness of *olive oil*, which contains about 10 per cent of glycerol in combination with fatty acids. The oil was given by stomach tube 4 to 6 hours before the injection of insulin, in order to allow a sufficient time for digestion. The data of table 3 clearly indicate that olive oil has a moderate protective action and the results would have been even more pronounced had it been possible to give an amount of oil equivalent to 6 grams of glycerol (60 grams of olive oil).

It should be pointed out that the antagonistic action of all the active substances showed itself not only in the prevention of death but also in the milder character of the symptoms, in fact it was noted that with the minimum protective dose of glycerol and alanin for instance, the symptoms were either absent or consisted only of depression.

The principal conclusion to be drawn from our experiments is that various carbohydrates, glycerol and at least one amino acid are physiological antagonists of insulin. This hormone therefore is not only strictly related to carbohydrate metabolism, but has relations to protein and fat metabolism. Whether these latter relations are direct or indirect cannot be determined on the basis of the present evidence. In all events it is advisable to regard the function of insulin in metabolism from a broad viewpoint.

SUMMARY

1. Various sugars are physiological antagonists of insulin: glucose, galactose, fructose, maltose, lactose, sucrose and trehalose. l-Arabinose and xylose have only a questionable activity. Inulin is inactive, due probably to lack of digestion.

2. Mannitol has a slight antagonistic action. Glycerol is very effective, both as a preventive of insulin death and as a curative agent. Glycerol decreases the hypoglycemia following insulin.

3. Lactic acid and pyruvic acid are inactive.

4. Glycin and glutaminic acid are inactive, whereas d-alanin is effective in about the same amount as glucose. Alanin decreases the hypoglycemia following insulin.

5. Olive oil, in the doses used, has a moderate activity, which is probably due to its glycerol component.

6. The physiological mechanism of insulin not only involves carbohydrate metabolism, but also that of the proteins and fats.

BIBLIOGRAPHY

(1) Banting, Best, Collip, Macleod and Noble: This Journal, 1922, lxii, 162, 559.
(2) Dakin: Physiol. Reviews, 1921, i, 394.
(3) Dakin and Dudley: Journ. Biol. Chem., 1914, xvii, 451.
(4) Herring, Irvine and Macleod: Biochem. Journ., 1924, xviii, 1023.
(5) Krogh and Lindhard: Biochem. Journ., 1920, xiv, 290.
(6) Lüthje: Deutsch. Arch. f. Klin. Med., 1905, lxxx, 101.
(7) Noble and Macleod: This Journal, 1923, lxiv, 547.
(8) Ringer and Lusk: Zeitschr. physiol. Chem., 1910, lxvi, 106.
(9) Thomas: Bull. Johns Hopkins Hosp., 1924, xxxv, 201.
(10) Voegtlin, Dunn and Thompson: Public Health Repts., 1924, xxxix, 1935.

CONTRIBUTIONS TO THE PHYSIOLOGY OF GASTRIC SECRETION

IV. The Stimulation of Gastric Secretion by Hydrolyzed Proteins[1]

A. C. IVY and A. J. JAVOIS

From the Hull Physiological Laboratory, The University of Chicago

Received for publication December 9, 1924

This research (1) was suggested by the observations of Ivy and McIlvain (2), who found that gastric secretion could be stimulated when certain substances were introduced into the intestine. Among such substances were the digestive products of fats and carbohydrates, one amino acid, several amines and peptone.

Part I. *Hydrolyzed proteins.* It has been known for some time that incompletely hydrolyzed proteins (Liebig's meat extract, Witte's peptone, Maggi soup cubes) stimulate gastric secretion when introduced into the stomach through a stomach tube or a gastrostomy (3), (4). Bickel found that meat and casein hydrolyzed by acid stimulates when administered by stomach tube. Schweitzer (5) confirmed the observations of Bickel and added hydrolyzed fish, liver and wheat.

Although their data show definite stimulation, the quantities of secretion collected were not very large. Further, they did not consider in their work the site ot action of the hydrolyzed products.

We have repeated and extended their observations, answering the following questions: 1, the effect of meat and casein hydrolyzed by acid; 2, the effect of casein hydrolyzed by "pancreatin"; 3, the effect of atropin on the resulting stimulation; 4, the effect of the hot water soluble fraction of meat hydrolyzed by acid; 5, the comparison of the effect of the hydrolyzed protein with the effect of the raw protein on gastric secretion; 6, the effect of subcutaneous injection of hydrolyzed meat and casein; 7, the effect of the oral administration of gastrin; 8, the site of action of casein hydrolyzed by "pancreatin."

Methods. Meat and casein were hydrolyzed by concentrated HCl, most of the acid being removed from the hydrolysate by distillation under reduced pressure. The end product was filtered and brought to volume

[1] This research has been aided by a grant from the Committee on Research of the American Medical Association.

with distilled water. The solution of all of the hydrolyzed products except one gave a faintly positive biuret reaction.

Casein was also hydrolyzed by "pancreatin" under toluol for from two to three weeks in an incubator, "pancreatin" being added daily and the reaction of the solution being maintained by the addition of the necessary amount of sodium carbonate. The final solution was reduced to volume under reduced pressure.

The water soluble fraction of meat was extracted by infusing it five times with boiling water. The resulting solution was then concentrated under reduced pressure and hydrolyzed by HCl.

The effect of the hydrolyzed protein was compared with the effect of the raw protein by the administration of aliquot portions.

Free acidity present in the hydrolyzed products was neutralized with sodium hydroxide prior to administration as it has been shown that free acid stimulates gastric secretion when applied to the duodenum (1). In some experiments the total acidity of the solutions administered in which the free acid was neutralized was usually 0.4 per cent, sometimes 0.6 per cent.

Pavlov pouch dogs were used and the solutions were given by stomach tube. Water controls were run as well as controls in which solutions of sodium chloride of the same concentration of chloride as found in the hydrolysates were used.

In this work forty experiments were done upon which we base our conclusions.

Results: The reader is referred to the tables for typical results.

Meat and casein hydrolyzed by HCl: Definite stimulation of gastric secretion resulted when hydrolyzed meat and casein were given by stomach tube, water and sodium chloride solution controls being negative or only slightly positive. The latent period of the stimulation varied from twenty to sixty minutes (table 1).

The amount of hydrolyzed meat administered was about two-sevenths of the amount of meat given (250 grams) as a test meal. We observed as much, sometimes much greater, stimulation from the hydrolyzed product of two-sevenths of the meal of meat as was observed when the entire meal was given unhydrolyzed. The same was true of casein. In most instances the period of stimulation was shorter when the hydrolyzed protein was given than when the raw protein was given.

Emesis and diarrhea frequently occurred on the administration of these hydrolyzed products. It occurred in most instances when the total acidity was neutralized. Vomiting occurred after a latent period of from 5 to 15 minutes, and diarrhea after a period of from 10 to 30 minutes. We have seen the hydrolyzed product passed per rectum within 15 minutes after the administration by stomach tube. When emesis or diarrhea occurred very little, if any, stimulation resulted.

Neutralization of the total acidity of the hydrolyzed product always resulted in a definite diminution in the response (table 1). It was difficult to make accurate observations on this point because of the frequent occurrence of emesis and diarrhea.

Different batches of the protein hydrolyzed by acid varied in their activity, although the method of hydrolysis remained the same.

Casein hydrolyzed by "pancreatin." This product never failed to stimulate gastric secretion, giving a uniform response, and did not cause emesis and diarrhea. This product was tried on a dog with a pouch of the entire

TABLE 1

Showing the effect of hydrolyzed meat given by stomach tube on gastric secretion

Pavlov pouch dogs

PROCEDURE	TIME	GASTRIC SECRETION			REMARKS
		Amount	Free acid*	Total acid*	
	o'clock	*cc.*			
Control	2–3	2.00	25	37	
200 cc. hydrolyzed meat (70 grams) at 3:00. Total acidity 0.4 per cent	3–4	2.0	45	62	Note latent period
	4–5	25.2	112	122	Pepsin 8 mm. by 1 cc.
	5–6	18.0	117	132	Pepsin 4 mm. by 1 cc.
	6–7	12.0	117	130	
Control	4–5	0.5	0	10	
200 cc. hydrolyzed meat (70 grams) at 5:00. Total acidity 0.4 per cent	5–6	12.0	97	112	Latent period 20 minutes
	6–7	4.5	125	135	
	7–8	6.5	122	130	
Control	2–3	1.7	0	15	
200 cc. hydrolyzed meat (70 grams) at 4:00. Solution neutral	3–4	2.3	0	15	
	4–5	3.0	25	50	
	5–6	3.0	35	60	
	6–7	2.5	35	60	

* Clinical units.

stomach and the duodenum anastomosed to the esophagus with positive results. Twelve grams of casein hydrolyzed by pancreatin gave a greater response than twelve grams of the raw casein (table 2). This product was also tried on an adult dog with a "denervated" pouch with all nerves cut except those in the walls of the blood vessels, with positive results.

Effect of atropin on the stimulation resulting from the administration of hydrolyzed proteins. The data in table 2 show definitely that atropin prevents the stimulation of gastric secretion by hydrolyzed proteins. This is important because it is well known that atropin prevents the gastric secretory response to a meal.

The hot water soluble fraction of meat. When the hot water soluble fraction of two pounds of meat was evaporated to dryness and then hydrolyzed by acid and administered, a slight stimulation of gastric secretion occurred.

TABLE 2

Showing effect of hydrolyzed casein given by stomach tube on gastric secretion

Pavlov pouch dogs

PROCEDURE	TIME	GASTRIC SECRETION			REMARKS
		Amount	Free acid*	Total acid*	
	o'clock	*cc.*			
Control	4–5	2.1	15	25	
100 cc. hydrolyzed casein (12 grams) at 5:00. Total acidity 0.6 per cent	5–6	5.3	70	95	
	6–7	10.0	117	130	
	7–8	8.0	110	120	
Control	4–5	2.0	0	10	
100 cc. hydrolyzed casein (12 grams) at 5:00. Solution neutral	5–6	3.0	35	57	
	6–7	4.0	100	115	
	7–8	3.0	75	95	
Control	11–12	3.5	0	10	
100 cc. pancreatinized casein (12 grams) at 12:00. Dog 39	12–1	11.0	60	75	
	1–3	3.4	42	62	
	3–4	2.0	0	12	
Control	12–1	2.0	0	10	Best response usually occurs 2nd or 3rd hour
100 cc. water, 12 grams casein at 1:00. Dog 39	1–2	3.0	50	60	
	2–3	2.0	55	65	
	3–4	0.8	35	50	
Control	12–1	1.3	0	10	
100 cc. pancreatized casein (12 grams) at 1:00	1–2	8.0	67	82	
	2–3	2.5	70	87	
1 mgm. atropin at 3:00	3–4	1.1	15	35	
	4–5	2.0	0	12	
100 cc. pancreatinized casein (12 grams) at 4:00. Dog 39	5–6	1.3	0	7	
	6–7	1.0	0	7	

* Clinical units.

The subcutaneous injection of hydrolyzed proteins. From 1 to 50 cc. of a slightly acid solution resulting from the acid hydrolysis of meat was injected subcutaneously with negative results. The large quantity was injected in only two experiments because a very severe local reaction resulted.

The site of action of casein hydrolyzed by "pancreatin." Hydrolyzed protein products may stimulate gastric secretion either by acting in the stomach, or in the intestine, or both.

In order to ascertain the site of action, dogs prepared with a pouch of the entire stomach and a duodeno-esophageal anastomosis were used (6).

From 20 to 30 cc. of the solution resulting from the "pancreatin" hydrolysis of casein were introduced into the stomach and returned again when ejected through the fistula, contact with, but without distention of, the gastric mucosa being continued for a period of thirty minutes. This procedure failed to stimulate the gastric glands, showing that the substances

TABLE 3

Showing the effect on gastric secretion of oral administration of gastrin and the site of action of hydrolyzed casein

PROCEDURE	TIME	GASTRIC SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	3–4	0.5	0	10	Pavlov pouch dog
50 cc. gastrin in 50 cc. water at 4:00	4–5	5.5	97	110	
	5–6	10.0	117	137	
	6–7	1.5	55	75	
Control	1–2	1.0	0	7	Dog with pouch of entire stomach. Solution applied was alkaline to litmus and acid to phenolphthalein
	2–2:30	0.5	0	7	
Applied to stomach 25 cc. "pancreatin" casein digest from 2:30–2:55	2:30–3	26.0	0	2	
	3–3:30	4.0	0	15	
	3:30–4	1.0	0	15	
	4–5	5.0	27	55	
Introduced into intestine 200 cc. of above digest equal to 6 grams casein at 4:00	5–6	4.8	52	72	
	6–7	2.0	35	52	
	7–8	1.8	17	32	
	8–9	3.1	5	12	

in hydrolyzed casein do not stimulate by contact with the gastric mucosa. In every experiment there was an increase in the quantity of mucus and a slight increase in the quantity of total acid, which could not be interpreted, however, as stimulation of gastric secretion.

When 100 to 150 cc. of the solution were introduced into the intestine, stimulation resulted out of all proportion to its water content, demonstrating that the substances in hydrolyzed casein stimulate via the intestine (table 3).

The oral administration of gastrin. The oral administration of 50 cc. gastrin by stomach tube (30 times the subcutaneous dose) caused a definite stimulation of gastric secretion (table 3).

Discussion. Our results prove definitely that hydrolyzed proteins stimulate gastric secretion when administered by stomach tube. This confirms the observations of Bickel and Schweitzer.

Our experiments on the site of the action of hydrolyzed protein solutions show that they stimulate by acting in or through the intestine and not in the stomach. It does not follow, however, that the stomach cannot be stimulated chemically by protein materials or their products. In fact, it has been shown by Lim, Ivy and McCarthy (7) that meat juice, β-alanine and histamine stimulate gastric secretion when applied to the gastric mucosa.

The observation that the hydrolyzed product of a fraction of the usual test meal of meat caused as much secretion as the entire test meal shows that the concentration and availability of the excitants is an important factor in the stimulation of gastric secretion. That the time of contact with the gastro-intestinal mucosa, or action in the intestine, is a basic factor is demonstrated by the fact that, if the solution caused diarrhea, stimulation did not result. This conclusion is corroborated by the results of Ivy, Lim and McCarthy on dogs with a duodeno-esophageal anastomosis and a pouch of the entire stomach, which showed that, if a fed meal passed through the intestine quickly, the gastric glands were not stimulated.

Why neutralization of the total acid of the hydrolyzed products resulted in a diminution of the response cannot be explained at present unless we assume that the exciting substances are changed or rendered less soluble. That some change did occur is suggested by the fact that such neutralized solutions were much more likely to cause diarrhea and emesis.

Our results on casein hydrolyzed by "pancreatin" show that when protein is hydrolyzed by a more natural process than acid hydrolysis abnormal symptoms do not result and more uniform stimulation of gastric secretion occurs. This strongly suggests that the two solutions or hydrolysates are chemically quite different. That such a hydrolyzed product acts in the intestine is obviously a very significant fact.

That the stimulation resulting from the administration of hydrolyzed proteins is prevented by subcutaneous injection of 1 mgm. of atropin shows that the stimulation is similar in nature to that caused by a meal as the stimulation following a meal is prevented by a similar dose of atropin.

Our observation on the effect of the oral administration of gastrin does not confirm the observation of Koch, Luckhardt and Keeton (8) who reported that gastrin by mouth does not stimulate gastric secretion. They do not record the amount of gastrin given, but on personal communication we have found that it was 10 cc. This explains the discrepancy as we found definite stimulation when 50 cc. of gastrin were given by

stomach tube. Since we have found that hydrolyzed proteins stimulate, there is no reason why gastrin should not stimulate unless the alcoholic extraction process used in the Koch method of preparing gastrin destroys the excitants, which it does not do as shown by our results.

The observation that subcutaneous injection of hydrolyzed meat and casein does not stimulate gastric secretion confirms the observations of Schweitzer (5), who found that hydrolyzed wheat, casein, liver, hay and fish were negative and gliadine doubtful, while hydrolyzed ereptone and spinach were positive. Some substances containing proteins on hydrolysis yield a gastric secretin, others do not. Since hydrolyzed meat, casein and other protein-containing substances stimulate when given by mouth, but not subcutaneously, it follows that the excitant in the protein hydrolysate is not a gastric secretin. Protein hydrolysates, then, must stimulate gastric secretion either by acting locally in the intestine or by containing substances that are converted into gastric secretins on assimilation. That they act on nerve endings in the mucosa is shown not to be the case because they stimulate a "denervated" stomach pouch. That they act on being absorbed or by elaborating a hormone does not seem to be tenable in the light of blood transfusion and cross-circulation experiments of Ivy, Lim and McCarthy (9). The remaining possibility is that they act by causing an increase in blood flow through the entire protal area by acting locally.

The cause of the emesis and diarrhea can only be explained by assuming that the particular hydrolysates of meat and casein contained substances that acted as marked irritants. It was not caused by the salt concentration, as controls on this point were negative. Since the complete analysis of such protein hydrolysates has not yet been made, we feel that this question cannot be answered at the present time.

CONCLUSIONS

1. Meat and casein hydrolyzed by acid stimulate gastric secretion when administered by stomach tube. The hydrolyzed product of two-sevenths of a test meal of meat is frequently adequate to call forth as much secretion as an entire test meal. Acid hydrolysates frequently cause vomiting and diarrhea when given by stomach tube.

2. Casein hydrolyzed by "pancreatin" stimulates gastric secretion uniformly and without causing symptoms. Stimulation of the glands of a "denervated" Heidenhain pouch occurs when hydrolyzed casein is given by stomach tube.

3. Atropin (1 mgm.) subcutaneously prevents stimulation by these hydrolysates.

4. The hot water soluble fraction of meat hydrolyzed by acid stimulates gastric secretion slightly.

5. Protein hydrolysates of meat and casein do not stimulate the gastric glands when injected subcutaneously.

6. Protein hydrolysates excite gastric secretion by acting via the intestine,—not in the stomach—, which we believe to be a significant fact.

7. The oral administration of gastrin (50 cc.) stimulates gastric secretion.

BIBLIOGRAPHY

(1) Ivy and Javois: Prelim. Rept., This Journal, 1924, lxviii, 132.
(2) Ivy and McIlvain: This Journal, 1923, lxvii, 124.
(3) Pavlov: The work of the digestive glands. London, 1910.
(4) Bickel: Intern. Beitr. z. Path. u. Therap. d. Ernährung, 1915, v, 73.
(5) Schweitzer: Biochem. Zeitschr., 1920, cvii, 256.
(6) Lim, Ivy and McCarthy: Quart. Journ. Exper. Physiol. (to be published), 1925, xv.
(7) Ivy, Lim and McCarthy: Ibid. (to be published).
(8) Koch, Luckhardt and Keeton: This Journal, 1920, lii, 508.
(9) Ivy, Lim and McCarthy: Quart. Journ. Exper. Physiol. (to be published), 1925, xv.

CONTRIBUTIONS TO THE PHYSIOLOGY OF GASTRIC SECRETION

V. The Stimulation of Gastric Secretion by Amino Acids[1]

A. C. IVY and A. J. JAVOIS

From the Hull Physiological Laboratory, The University of Chicago

Received for publication December 2, 1924

As no thorough study has been made—up to the present work— on the effect of amino acids (1) on gastric secretion, the literature is meager and scattered. The work that has been done will be referred to specifically as each amino acid is taken up.

Bickel (2) concludes that amino acid mixtures of various origin, when introduced into the stomach, act as powerful excitants of ga tric secretion. Schweitzer (3) states that this is not a common property of the amino acids. These statements are based for the most part on results obtained with protein hydrolysates. Bickel did not use pure amino acids, but referred to the work of Ehrmann (4) who used alanine and glycocoll. Schweitzer used only one pure amino acid. Since the exact composition of protein hydrolysates is not known, we do not believe that a comparison between protein hydrolysates and pure amino acid mixtures can be made with accuracy.

In this work we have studied each amino acid in varying doses and a mixture of them in the concentration found in casein. We have used all the amino acids available on the market and in as pure a form as they could be obtained.

Methods. Pavlov pouch dogs were used in most of the experiments. In some experiments Heidenhain pouch dogs, "denervated" pouch dogs and dogs with a pouch of the entire stomach and a duodeno-esophageal anastomosis were used.

Amino acids with free acid neutralized before injection were administered by stomach tube and hypodermically. Only one was administered intravenously for the reason that it was the only one that gave suggestive responses when injected subcutaneously. The amino acids that were not soluble were suspended in a small amount of water and washed down the tube with an additional quantity, the total amount of water being kept

[1] This research has been aided by a grant from the Committee on Research of the American Medical Association.

below 50 cc. A hypodermic needle with a large bore was used, if the amino acid was insoluble.

A sufficient number of "water-control" experiments were done to show that our results were not due to water stimulation.

In our first experiments small doses of the various amino acids were used (0.050 to 0.1 gram), but when only negative results were obtained larger doses were administered.

TABLE 1

Summary of results on the effect of amino acids on gastric secretion

SUBSTANCE	DOSE	NUMBER OF EXPERIMENTS	RESULTS		
			Positive	Negative	Doubtful
	grams				
1. Glycine	1.5 –2.5	4	4	0	0
2. α-Alanine	1–1.5	7	0	5	2
	2.5	2	2	0	0
3. β-Alanine	1–1.5	8	7	0	1
4. Iso-valine*	0.2–2.0	11	1	7	3
5. Tryptophane	0.15–0.5	5	0	5	0
	0.6–1.0	4	2	1	1
6. Phenylalanine	1–2.5	5	2	3	0
7. Histidine dichloride	1	5	1	2	2
8. Tyrosine	1–2	12	3	7	2
9. Iso-leucine	1–2	4	0	4	0
10. Leucine	1–2	12	4	8	0
11. Glutannic acid	1–3	7	0	5	2
12. Cystine	1–2	9	2	5	2
13. Cysteine HCl	0.5–1	5	0	5	0
	1.5–2.5	3	3	0	0
14. Asparagin	1.5	4	2	1	1
15. Aspartic acid	1.0–1.5	4	2	1	1
16. Lysine picrate†	1	2	2	0	0
17. Arginine	1	4	1	3	0
18. dl-valine	1	4	0	3	1
19. α-amino-n-caproic acid‡	1	8	3	3	2
20. dl-α-amino-n-butyric acid	0.2–2.0	11	1	7	3
21. Mixture of amino acids		13	7	4	2

* dl-α-amino-α-methylbutyric acid.

† Stimulation due to picrate ion.

‡ Nor-leucine or caprine.

The routine experimental procedure consisted as follows: 1, to collect the continuous secretion of two or three one hour periods for control; 2, to administer the substance under study; 3, to collect the secretion for three or more hours; and 4, to give a meal or some known stimulant of gastric secretion, if no response occurred from the substance administered. This was done in order to prove that the gastric glands were responsive at the time of experimentation.

In our tables we only show one hour of continuous secretion and report no responses of our animals to meals, for the sake of brevity.

We desire to emphasize that only dogs were used that were known to secrete well to a meal, that is, from 10 to 30 cc. of gastric juice per hour.

Amino acids and amines used in this study have been bought from the Special Chemicals Company and Eastman Kodak Company. Because of the time-consuming nature of our problem, it was impossible for us to study any of the amino acids not on the market or to check the purity of the products we have used.

RESULTS. We have classified our results as positive, negative and doubtful. We have termed a result doubtful when it might be accounted for by the not unusual variation in the amount and acidity of the continuous secretion or by water stimulation, which are points that must be considered in work of this nature. One or two doubtful results are shown in the tables, so that the reader may know what we term a doubtful result. The results recorded in the table are in most instances the best response observed for the substance under study.

Glycine. Ehrmann (4) found that the subcutaneous injection of from 0.5 to 1.0 gram of glycocoll had no effect on gastric secretion. This we have confirmed.

Ivy and McIlvain (2) failed to observe stimulation of gastric secretion when a 2 per cent glycocoll solution was applied to the mucosa of a Thiry's fistula of the duodenum. It was found later by one of us (Ivy) that the glycocoll used in this work was impure and was negative when given by mouth.

We have found that glycine (Pfanstiehl) stimulates gastric secretion when given in doses of 1.5 grams or more (table 2).

Subcutaneously glycine is inactive in doses as large as 0.5 gram.

α-alanine. Ehrmann (4) injected from 0.5 to 1.0 gram of alanine subcutaneously with negative results. This we have confirmed.

We have found that when 2.5 grams. of α-alanine (Pfanstiehl) are given by stomach tube stimulation occurs (table 2).

β-alanine. β-alanine (Pfanstiehl) is the most active gastric secretory excitant of the amino acids that we have had an opportunity to use. Half a gram administered by stomach tube is usually negative, one gram is always positive and one and a half gram always excites a decided secretion of gastric juice, if the gastric secretory mechanism is normal. The maximum secretion usually appears the first hour after administration and frequently, if 1.5 gram is administered, the secretion is equivalent in quantity and quality to that excited by a meal of meat. This action of β-alanine is inhibited by 1 mgm. of atropin (table 2).

We have injected β-alanine subcutaneously in doses of from 0.5 to 1.0 gram with doubtful results. Doses under 0.5 gram were definitely nega-

tive. Three slightly positive results have occurred when a dose of 1 gram was used (tables 2 and 6). This dose is quite inconstant in its effect and

TABLE 2

Effect of amino acids on gastric secretion

Pavlov pouch dogs

PROCEDURE	TIME	SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	2–3	1.7	0	32	
2.5 grams glycine at 3:00 per os in	3–4	4.5	57	85	
40 cc. water	4–5	3.0	42	75	
	5–6	2.5	35	60	
Control	3–4	0.7	0	0	
2.5 grams cysteine at 4:00 per os	4–5	4.1	37	65	
in 40 cc. water	5–6	3.5	75	100	
	6–7	1.0	50	62	
Control	1–2	2.5	0	12	
1.5 grams β-alanine at 2:00 per os	2–3	11.0	80	107	
in 30 cc. water	3–4	3.0	32	75	
Control	12–1	3.7	0	17	
0.5 gram β-alanine* subcutaneously	1–2	3.6	0	20	Dog 19
at 1:00 1 gm. β-alanine per os at	2–3	2.0	0	15	
3:00	3–4	6.0	45	75	
	4–5	2.0	30	60	
Control	1–2	0.6	0	20	
1 gram β-alanine subcutaneously at	2–3	2.1	50	85	Dog 23
2:00 1 gram β-alanine per os at	3–4	2.5	27	67	
4:00	4–5	6.0	95	122	
	5–6	2.8	67	95	
Control	2–3	1.0	12	35	
2.5 grams α-alanine at 3:00 per os	3–4	4.1	37	70	
in 30 cc. water	4–5	2.3	62	90	
	5–6	1.2	30	45	
Control	5–6	1.5	0	10	
2.5 grams phenylalanine at 6:00 per	6–7	3.6	70	85	
os in 40 cc. water	7–8	1.0	62	77	
	8–9	0.9	57	67	

β-alanine intravenously is negative.

has never stimulated to the extent that 1 gram per os stimulates. (We have been unable to settle this point without question because we have been able to secure only a limited supply of β-alanine.)

Intravenously 0.5 gram of β-alanine has no effect on gastric secretion or the objective condition of the animal.

β-alanine stimulates gastric secretion when acting both in the stomach and intestine (6). This has been demonstrated by using a dog with a pouch of the entire stomach and a duodeno-esophageal anastomosis.

iso-valine. dl-α-amino-α-methylbutyric acid (Eastman) was administered by stomach tube in doses of from 0.2 to 2.0 grams. Only one positive result occurred. Two grams were given in two experiments with doubtful results. So we believe that iso-valine is either a very mild or uncertain excitant of the gastric glands (table 4).

Subcutaneously it has no effect in 0.4 gram doses.

Tryptophane. Tryptophane (Pfanstiehl) when given in 1 gram doses by stomach tube definitely stimulated gastric secretion in two experiments (table 3). Smaller doses yielded only negative or doubtful results.

Subcutaneously (300 mgm.) it was consistently negative. Koch, Luckhardt and Keeton (7) found that 20 mgm. subcutaneously did not stimulate.

Phenylalanine. Phenylalanine (Pfanstiehl) is a feeble and an uncertain excitant of gastric secretion when given by stomach tube in doses of from 1.0 to 2.5 grams (table 2).

Subcutaneously (200 mgm.) it is negative.

Histidine dichloride. Koch, Luckhardt and Keeton (7) injected 10 mgm. of histidine subcutaneously without stimulating gastric secretion. Komaroff (8) reported recently that histidine has a slight stimulating action when injected. We were unable to ascertain the dose he used.

We have injected from 10 to 150 mgm. of histidine dichloride subcutaneously without stimulating the gastric glands and are at a loss to explain Komaroff's statement unless he failed to take into account the continuous secretion.

In a preliminary report (1) we stated that histidine (Pfanstiehl) was negative per os in 1 gram doses. Since making that report an experiment has been performed in which definitely positive results were obtained (table 3). This lack of constancy shows that it is either a feeble excitant or whether or not it stimulates depends on conditions in the gastro-intestinal tract which vary, e.g., the flora.

Tyrosine. Ivy and McIlvain (5) found that when 0.5 gram of tyrosine (Pfanstiehl) was applied (a 0.3 per cent solution in water) to a Thiry's fistula of the duodenum stimulation of gastric secretion frequently resulted.

We have found that when 1 gram is given by stomach tube no stimulation occurs usually, but a definite stimulation does occur in some cases after a latent period of one hour (table 3). We have observed stimulation

of gastric secretion to occur when 1 gram was given per os to a dog with a pouch of the entire stomach and a duodeno-esophageal anastomosis.

No stimulation occurs when doses as large as 300 mgm. are injected subcutaneously.

iso-leucine. iso-leucine (Pfanstiehl) does not stimulate gastric secretion when administered subcutaneously (500 mgm.) or by stomach tube (1 to 2 grams). This amino acid was used in only four experiments, but since it was used in 2-gram doses in two of these experiments with negative results, we believe we are warranted in doubting it to be a gastric secretory excitant.

Leucine. Leucine (Pfanstiehl) when administered by stomach tube in doses of from 1 to 2 grams is a definite, but an inconstant, excitant of gastric secretion (table 3).

Subcutaneously in doses of 200 mgm. it was ineffective.

Glutamic acid. Glutamic acid (Pfanstiehl) was consistently negative when given by stomach tube even in 3 gram doses. We believed that such a large dose was indicated for trial because of the high percentage of glutamic acid in some proteins.

It has no effect when injected subcutaneously in 200 mgm. doses. Schweitzer (3) injected from 0.5 to 1.0 gram subcutaneously with negative results.

Cystine. Cystine (Pfanstiehl) by stomach tube in doses of from 1 to 2 grams was slightly and inconstantly positive (table 3).

Koch, Luckhardt and Keeton (7) injected 20 mgm. of cystine subcutaneously and observed one positive, two negative and one doubtful result. We have injected 350 mgm. with the results shown in table 7. One definitely positive result was observed with a latent period of two hours. Because of the length of the latent period we question whether this was the effect of the cystine, although we were unable to account for it in any other way. Our unsatisfactory results are quite like those of Koch, Luckhardt and Keeton.

Cysteine hydrochloride. Cysteine (Pfanstiehl) by stomach tube in doses of 1 gram or less failed to stimulate gastric secretion, but in doses of 1.5 and 2.0 it stimulated in every experiment (table 2).

Cysteine proved to be ineffective when injected subcutaneously (500 mgm.).

Asparagin. Ivy and McIlvain (5) applied 0.75 per cent asparagin solution (100 cc.) to the mucosa of a Thiry's fistula with negative results.

Our results show that if 1.5 grams is given by stomach tube a slight stimulation of gastric secretion occurs (table 3).

Subcutaneously asparagin is negative in doses as large as 500 mgm.

Aspartic acid. Aspartic acid (Pfanstiehl) given by stomach tube in 1 and 1.5 grams doses was slightly positive (table 4).

TABLE 3

Effect of amino acids on gastric secretion

Pavlov pouch dogs

PROCEDURE	TIME	SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	2–3	2.0	0	10	
1 gram tryptophane at 3:00 per os in 30 cc. water	3–4	4.4	52	70	
	4–5	2.2	60	82	
	5–6	2.0	35	55	
	6–7	1.0	12	20	
Control	11–12	0.7	0	10	
1 gram histidine at 2:00 per os in 30 cc. water	12–2	1.3	10	22	
	2–3	2.5	17	27	Called a doubtful result
	3–4	1.8	20	27	
	4–5	1.8	17	22	
Control	2–3	0.5	0	0	
1 gram histidine at 3:00 per os in 40 cc. water	3–4	5.8	45	75	
	4–5	4.5	52	77	Excellent dog
	5–6	3.2	32	52	
Control	2–3	1.0	22	35	
1 gram tyrosine at 3:00 per os in 50 cc. water	3–4	0.7	22	27	
	4–5	4.0	70	82	
	5–6	1.5	70	82	
Control	9–10	1.7	17	42	
1 gram leucine at 11:00 per os in 40 cc. water	10–11	2.0	12	40	
	11–12	4.0	40	75	
	12–1	2.2	10	77	
Control	1–2	1.0	10	42	
2 gram cystine at 2:30 in 30 cc. water	2–3	0.8	5	25	
	3–4	2.5	40	82	
	4–5	1.7	52	75	
	5–6	2.0	47	62	
Control	1–3	2.5	17	55	
1.5 gram asparagin at 3:00 Repeated at 4:00	3–4	3.5	30	62	
	4–5	4.0	40	75	
	5–6	3.0	37	70	

Subcutaneously aspartic acid is negative in doses of 200 mgm.

Lysine picrate. We have been unable to obtain lysine in any other form than the picrate. One gram of lysine picrate by stomach tube stimulates

TABLE 4

Effect of amino acids on gastric secretion

PROCEDURE	TIME	SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	2–3	2.1	0	25	
1 gram glutamic acid at 3:00 per os in 30 cc. water	3–4	2.1	15	32	Doubtful
	4–5	1.7	35	52	
	5–6	1.5	47	62	
Control	10–11	1.6	32	67	
0.5 gram iso-valine at 11:00 per os in 20 cc. water	11–12	3.0	60	82	
	12–1	3.5	50	70	
	1–2	2.5	32	57	
Control	8–9	3.0	12	42	
1 gram amino caproic acid at 10:00 per os suspended in 30 cc. water	9–10	2.5	7	37	
	10–11	6.2	50	77	
	11–12	2.5	50	77	
	12–1	1.4	7	45	
	1–2	1.0	0	12	
Control	2–3	1.2	0	42	
1.5 gram aspartic acid at 3:00 in 30 cc. water	3–4	3.3	20	62	Slight stimulation
	4–5	1.6	10	37	
	5–6	2.2	0	27	
Control	11–12	3.2	27	70	
1 gram arginine at 12:00 in 30 cc. water	12–1	5.0	40	85	
Meal at 3:00	1–2	1.5	37	80	
	2–3	2.0	32	52	
	3–4	5.5	40	77	
Control	2–3	1.4	0	7	
1 gram lysine picrate in 40 cc. water at 3:30	3–4	1.7	0	7	
	4–5	4.0	85	100	
	5–6	1.0	75	90	
Control	1–2	3.5	30	60	
2 gram α-amino-n-butyric acid in 20 cc. water at 2:00 50 cc. water at 5:00	2–3	5.0	40	75	
	3–4	4.3	40	75	
	4–5	2.5	85	92	
	5–6	3.0	47	75	
Meal at 7:00	6–7	0.5	15	25	
	7–8	6.0	47	77	

quite markedly (table 4). But we have found that 0.5 gram of picric acid, free acidity neutralized, stimulates to the same degree. So we believe that the exciting agent in lysine picrate is the picrate ion.

Arginine. Arginine (Pfanstiehl) was administered four times in doses of 1 gram with one positive result. In this experiment (table 4) the gastric glands were quite active at the time the arginine was given, under which condition the glands are more readily stimulated. Because of the three negative results we can only conclude that arginine is a very mild stimulant of the gastric glands.

Valine. dl-valine (Eastman) failed to stimulate gastric secretion when given by mouth (1 gram) or subcutaneously (200 mgm.)

α-Amino-n-caproic acid, or nor-leucine. Amino caproic acid (Eastman) when given by stomach tube in 1 gram doses definitely, but inconstantly, stimulated gastric secretion (table 4).

Subcutaneously it is negative (400 mgm.).

dl-α-amino-n-Butyric acid. When from 0.2 to 1.0 gram of amino butyric acid (Eastman) was given by stomach tube, stimulation never occurred; but positive and doubtful results were obtained, when the dose was increased to 2.0 grams.

Since it is known that butyric acid stimulates gastric secretion (5), it is possible that the stimulation observed here is due to butyric acid.

Subcutaneously its action is doubtful (tables 6 and 7).

A mixture of amino acids—"synthetic hydrolyzed casein"? The mixture of amino acids that we have used is based upon the analysis of casein. We used quantities of amino acids that would result from the hydrolysis of 12.5 grams of casein, it being kept in mind that only approximately 66 per cent of casein has been accounted for in the form of amino acids. We chose 12.5 grams of casein for the reason that that was the quantity of hydrolyzed casein we used in the work described in the preceding paper. We chose this quantity as it is about the quantity of casein in a pint of milk. The following were the amino acids used in our mixture: glycine, 0.060 gram; α-alanine, 0.25 gram; leucine, 1.25 grams; phenylalanine, 0.40 gram; tyrosine, 0.50 gram; cystine, 0.020 gram; aspartic acid, 0.20 gram; glutannic acid, 2.00 grams; tryptophane, 0.20 gram; arginine, 0.60 gram; histidine, 0.30 gram; valine, 1.00 gram. This makes a total of 7.58 grams of amino acids at one dose. The mixture still lacked serine (0.06 gram), proline (1.00 gram) and oxyproline (0.04 gram) of being complete according to data given by Plimmer (11) on the analysis of casein. Of course it lacked other unknown substances that are present in hydrolyzed casein solutions.

The above mixture was put into 50 cc. of warm water and given to Pavlov pouch dogs by stomach tube, being washed down by an additional 50 cc. of water. The experimental procedure outlined above was followed. Only dogs were used that were known to secrete well.

Eight experiments were performed with the above mixture and five from which aspartic acid and arginine were omitted. Of the thirteen

TABLE 5

Effect of a mixture of amino acids—"Synthetic hydrolyzed casein"?—given by mouth on gastric secretion

PROCEDURE	TIME	GASTRIC SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	1–2	0.5	0	12	
Control	2–3	1.0	0	12	
Amino acid mixture at 3:00, 60	3–3:30	1.0	12	37	Dog "M," pouch dog
cc. water	3:30–4:30	5.0	60	87	
	4:30–5:30	2.3	60	85	
Control	11:30–12:30	2.2	10	47	
Control	12:30–1:30	2.8	10	45	
Amino acid mixture at 1:30	1:30–2:30	2.3	15	45	
100 cc. water	2:30–3:30	3.0	45	62	Dog "P," pouch dog
	3:30–4:00	2.0	45	57	
	4–5	7.4	82	95	
	5–6	5.5	100	112	
	6–7	3.5	112	137	
Control	11–12	1.5	7	35	
Control	12–1	2.0	5	35	
Amino acid mixture at 1:00	1–2	3.5	7	47	Dog "D," pouch dog
100 cc. water	2–3	2.5	7	42	
	3–4	3.0	5	42	
	4–5	4.5	20	50	
	5–6	4.2	87	95	
	6–7	3.0	57	77	
	7–8	3.5	50	60	
Control	11:30–12	2.5	0	10	
Control	12–12:30	2.0	0	10	
Control	12:30–1	2.0	0	10	Dog "R." Dog be-
Introduced into intestine ami-	1–1:30	3.0	0	20	came quite sick at
no acid mixture at 1:40 min-	1:30–2	2.9	27	52	2:10 and vomited
us arginine and valine. 80	2–3	0.5	27	52	bile-stained fluid
cc. water	3–4	7.2	27	52	
Dog "R"—pouch of entire	4–5	8.3	37	60	
stomach and duodeno-	5–6	8.5	37	60	
esophageal anastomosis	6–7	7.5	42	65	

experiments seven were positive, four negative and two doubtful (table 1). In two of the four negative experiments, for some unknown reason,

the dog failed to respond to a meal four hours after giving the amino acids. The two doubtful experiments might be classed as negative, because of the quantity of water that had to accompany the administration of the mixture.

Our results show that the amino acid mixture comparable to hydrolyzed casein stimulates gastric secretion, but not to the extent nor with the regularity that hydrolyzed or "pancreatinized" casein does (table 5).

It should be pointed out that in the mixture used there is only one of the amino acids that stimulates gastric secretion present in sufficient quantity to act and that is leucine, which is an inconstant excitant. In view of this fact, we were not entirely surprised, although somewhat disappointed, with our results.

TABLE 6

Effect of subcutaneous injection of three doubtfully active amino acids

Pavlov pouch dogs

PROCEDURE	TIME	GASTRIC SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	4–5	0.7	0	0	
1 gram β-alanine at 5:00 subcutaneously	5–6	2.0	30	45	See table 2
	6–7	3.0	55	67	
Repeated at 6:00	7–8	2.8	62	75	
Control	6–7	1.0	12	20	
0.35 gram cystine at 7:00 subcutaneously	7–8	2.5	20	37	
	8–9	2.0	37	57	
	9–10	6.0	70	87	
Control	12–1	1.8	0	32	
0.4 gram dl-α-amino-n- butyric acid subcutaneously at 2:00	1–2	2.0	0	25	
	2–3	2.5	10	55	
	3–4	3.2	5	45	
	4–5	2.0	10	40	

Our result following the administration of the amino acid mixture minus arginine and valine to a dog with a pouch of the entire stomach and a duodeno-esophageal anastomosis shows that amino acids stimulate from the intestine. The quite violent vomiting that results strongly suggests that such a concentrated mixture has an irritating effect on the duodenum. This is especially interesting in view of the fact that we never observed vomiting when the amino acid mixture was introduced into the stomach.

Addendum: It should be stated that in some experiments there occurred following the oral administration of some amino acids a two- to fourfold increase in the mucus secreted by the pouch along with an increase in pepsin content without any increase in acid. Although this was a very

interesting and significant observation, our supply of amino acids was too limited to permit further investigation of this phenomenon. We did not interpret this phenomenon as a positive result. We believe, however, that it signifies a very mild type of stimulation because we have frequently observed in the course of other work on gastric secretion that prior to the secretion of gastric juice there is an increased secretion of mucus. For example, when a pouch dog after ingesting a meal does not secrete gastric juice the first hour, there frequently occurs an augmentation in the quantity of mucus secreted.

TABLE 7

Summary of experiments showing the effect of three doubtfully active amino acids when injected subcutaneously

SUBSTANCE	DOSE	NUMBER OF EXPERIMENTS	RESULTS		
			Positive	Negative	Doubtful
	grams				
β-alanine	0.5–1	8	3	5	0
Cystine	0.35	5	1	3	1
dl-α-amino-n-butyric acid	0.3–0.5	8	0	3	5

SUMMARY AND DISCUSSION

β-alanine is a marked excitant of gastric secretion. Glycine and cysteine hydrochloride are definite and regular excitants, whereas α-alanine, tyrosine, leucine, phenylalanine, asparagin, nor-leucine, arginine, tryptophane and aspartic acid are feeble and inconstant excitants of gastric secretion when given by stomach tube. Cystine, histidine, amino butyric acid, iso-valine have yielded doubtful or inconclusive results. Iso-leucine, dl-valine and glutamic acid are definitely negative. The dose must be relatively large—usually from one to two grams.

None of the amino acids studied are gastric secretins in the sense that when injected subcutaneously they excite gastric secretion. Our results force us to suspect β-alanine, but the quantity required and the small and inconstant response force us to the conclusion that it is not a gastric secretin. This is significant in that it shows that none of the twenty amino acids used by us can be the active constituent of gastrin.

We are unable to suggest any relation between the chemical structure or properties and the gastric secretagogue action of the active amino acids. We have used the theoretical decomposition products of β-alanine and have found some of them active. The data obtained will be given in our succeeding paper.

The fact that some of the amino acids act only after a long latent period, one hour or more, and are not constant in action strongly suggests that they do not act per se, but act after being changed in some way. The most reasonable assumption that has occurred to us is that they are converted

into their amine or aporrhegma before acting. This is reasonable on the basis of our work on the amines and aporrhegmas (see succeeding paper) and of the fact that the flora of the gastro-intestinal tract are variable.

We do not believe that this or any of our observations might be due to impurities as the analysis of the amino acids furnished by the manufacturer (Special Chemicals Company, Highland Park, Ill.) shows that they are all about 100 per cent pure with the exception of iso-leucine, β-alanine and arginine, which, however, analyze approximately 99 per cent pure.

Our observation that an amino acid mixture is not as potent as and acts in a manner different from hydrolyzed casein, hydrolyzed by acid or digested with "pancreatin," can best be explained by assuming that hydrolyzed casein contains either polypeptids or other substances which are more potent gastric secretory excitants than the amino acids with the possible exception of β-alanine. It is also quite possible that prepared amino acids may not be nascent.

The significance of our studies on the amino acids in relation to the digestive processes as they occur normally is difficult to evaluate because of our lack of knowledge of their rate of formation and the quantity present at any one time in the gastro-intestinal tract. For example, when casein is digested in the intestine without passage through the stomach, stimulation of gastric secretion occurs (10), but it is impossible for us to say whether this is due to the action of polypeptids, amino acids or amines, because we are not acquainted with the quantitative changes that occur in digesting casein in the intestine. However, on comparing our results on hydrolyzed casein, the amino acid mixture and the amines, we are inclined to believe that polypeptids, amino acids and amines are all concerned in the normal process.

We were unable to obtain any evidence that any of the amino acids exerted an inhibitory effect upon gastric secretion.

The amino acids excite gastric secretion when acting via the intestine. β-alanine also excites when in contact with the gastric mucosa. Whether or not other amino acids act in the stomach, we cannot say as further experiments on that point were not done.

BIBLIOGRAPHY

(1) Ivy and Javois: Prelim. Rept., This Journal, 1924, lxviii, 132.
(2) Bickel: Intern. Beitr. z. Path. u. Therap. d. Ernährung, 1915, v, 73.
(3) Schweitzer: Biochem. Zeitschr., 1920, cvii, 256.
(4) Ehrmann: Intern. Beitr. z. Path. u. Therap. d. Ernährung, 1912, iii, 382.
(5) Ivy and McIlvain: This Journal, 1923, lxvii, 124.
(6) Lim, Ivy and McCarthy: Quart. Journ. Exper. Physiol., 1925, xv.
(7) Koch, Luckhardt and Keeton: This Journal, 1920, lii, 508.
(8) Komaroff: Physiol. Abst., 1924, viii, Abst. no. 364.
(9) Plimmer: The chemical constitution of proteins. Part 1, London, 1917.
(10) Ivy, Lim and McCarthy: Quart. Journ. Exper. Physiol. (to be published), 1925, xv.

CONTRIBUTIONS TO THE PHYSIOLOGY OF GASTRIC SECRETION

VI. The Stimulation of Gastric Secretion by Amines and Other Substances[1]

A. C. IVY and A. J. JAVOIS

From the Hull Physiological Laboratory, The University of Chicago

Received for publication December 2, 1924

Since it is known that amines occur normally in the intestinal tract and that the intestine serves as a path for stimulation of the gastric glands, it is quite possible that the amines might serve as gastric secretory excitants and account in part for the intestinal phase of gastric secretion and play a rôle in the genesis of the continuous secretion of the gastric glands.

Histamine. *Subcutaneously:* It is well established that histamine stimulates gastric secretion when injected subcutaneously in a dose of from 0.5 to 1.0 mgm.

Per os: Koskowski (1) has reported that the gastric glands of pigeons are stimulated when large doses of histamine are introduced into the intestine. Popielski (2) introduced 3.2 mgm. into the duodenum with no effect on gastric secretion. Rothlin and Gundlach (3) found that the intestinal application of histamine (1.0 mgm.) does not stimulate the gastric glands. Ivy and McIlvain (4) observed that the application of 1:1000 solution of histamine to the mucosa of a Thiry's fistula of the intestine causes a marked secretion of gastric juice in pouch dogs. Ivy, McIlvain and Javois (5) observed that when histamine was given by stomach tube to a man (225 mgm.) and dog (100 mgm.) gastric secretion is stimulated. Lim, Ivy and McCarthy (6) have observed that local application of histamine solution to the gastric mucosa of dogs stimulates the gastric glands.

The data presented in tables 1 and 3 definitely establish the fact that histamine on gastro-intestinal administration in large doses (50-100 mgm.) stimulates gastric secretion.

Intravenously: Popielski (2) reported that the intravenous injection of 0.8 mgm. of histamine within one minute does not stimulate gastric secretion. (His animal showed marked symptoms.) Rothlin and Gundlach (3) reported that histamine had no effect on the gastric glands when

[1] This research has been aided by a grant from the Committee on Research of the American Medical Association.

given intravenously. They injected from 0.4 to 0.05 mgm. without regard to time. Lim (7), performing acute experiments on cats weighing about 3 kilos, observed that when from 1 to 3 cc. of 0.0001 per cent histamine solution were injected intravenously the gastric glands were stimulated slightly. He accounted for this discrepancy with the above observers by stating that the difference might be due to the fact that in his experiments the cat had the abdominal vagi severed and was under an anesthetic.

It occurred to us that the discrepancy in the above results is due to the rate of injection. None of the above investigators have taken into con-

TABLE 1

The effect of amines administered by mouth on gastric secretion. Summary

Pavlov pouch dogs

SUBSTANCE	DOSE	NUMBER OF EXPERIMENTS	RESULTS		
			Positive	Negative	Doubtful
Histamine (dog)	50–150 mgm.	Many	All	0	0
Histamine (man)	100–225 mgm.	3	3	0	0
Epinine	0.15–1 gram	6	6	0	0
Ethylamine HCl	0.5 –1.5 grams	8	5	2	1
Methylamine HCl	1–1.5 grams	7	6	1	0
n-Amylamine	1 cc.	3	2	1	0
iso-Amylamine	1–2 cc.	8	3	4	1
Tyramine phosphate	0.15–1 gram	9	0	7	2
Adrenalin	1:3,000 1:10,000	10	0	8	2
Hydroxylamine* HCl	0.25–1 gram	5	0	5	0
Glycosamine	1 gram	3	0	3	0
Pyrolidine	1 cc.	4	4	0	0
p-Phenylenediamine	0.2–0.5 gram	3	0	3	0
iso-Propylamine	0.3–1 cc.	7	5	1	1
Ethyl-methylamine HCl	0.4–1 gram	8	7	1	0

* Phenylhydroxylamine is very toxic. Hydroxylamine inhibits.

sideration the slow rate of passage of histamine from muscular or subcutaneous tissue into, and its great dilution in, the blood stream and have injected histamine intravenously at a rapid rate. The dose used by Lim approximates more closely the conditions incident to absorption from subcutaneous tissue than the dose of the other investigators and we are not surprised that Lim observed slightly positive results.

We have injected histamine intravenously in a low concentration at a slow rate (table 2). We have found that a definite stimulation results in a Pavlov pouch when 1 mgm. of histamine in 30 cc. of sodium chloride solution was injected at a rate of 1 cc. per minute. The dog

weighed 12 kilos, the dose being 0.0027 mgm. per kilo per minute. When the injection rate was increased to 0.0033 mgm. per kilo per minute, a greater stimulation occurred. This dosage caused no symptoms except defecation in one animal and slight dilatation of the skin vessels in another

TABLE 2

Effect of intravenous timed injection of histamine and adrenaline on gastric secretion

PROCEDURE	TIME	GASTRIC SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Dog "B" 12 kilos	10:30–11	0.8	0	15	
1 mgm. histamine in 30 cc. NaCl	11:00–11:30	0.9	0	15	
solution given intravenously at	11:30–12:00	0.3	0	12	Defecation at 12:40
a rate of 1 cc. per minute from	12:00–12:30	1.6	2	35	
12:00–12:30	12:30–12:45	2.1	60	77	No symptoms
	12:45–1:00	0.8	60	77	No vasodilatation
Total histamine given 1 mgm.	1:00–1:30	0.5	25	45	of skin vessels
	1:30–3:00	1.5	10	20	Latent period 20
0.0027 mgm. per kilo per minute	3:00–3:30	1.4	0	17	minutes
	3:30–5:00	1.4	17	30	Normal response in
1 mgm. histamine subcutaneously	5:00–6:00	1.2	20	45	one hour 6 cc.
at 3:40					
Dog "P" 10 kilos	1:00–1:30	0.5	0	7	
1 mgm. histamine in 30 cc. NaCl	1:30–2:00	0.8	0	10	
solution given intravenously at	2:00–2:30	0.7	0	12	
a rate of 1 cc. per minute from	2:30–3:00	0.8	0	12	No symptoms
3:00–3:25	3:00–3:30	5.0	62	77	Only slight vaso-
	3:30–3:45	2.6	92	115	dilatation of skin
Total histamine given—	3:45–4:00	0.8	80	97	vessels
0.833 mgm. 0.0033 mgm. per kilo	4:00–5:00	1.5	22	60	Latent period 15
per minute					minutes
Control	12:00–1:00	2.5	12	27	
Control	1:00–2:00	1.8	7	20	
10 cc. of adrenalin, 1:200,000 at	2:00–3:00	4.0	12	30	
2:00 in 120 seconds	3:00–4:00	1.8	2	12	
10 cc. of 1:200,000 at 4:00 in 120	4:00–4:30	2.8	27	42	
seconds	4:30–5:00	2.0	30	47	
	5:00–6:00	2.0	5	25	

(table 2). The latent period of stimulation seems to vary with the rate of injection and is longer than on subcutaneous injection. Sodium chloride solution controls were negative.

According to Koessler and Hanke (8), 0.0027 mgm. per kilo per minute

is the smallest dose of histamine that will cause a fall in blood pressure. The fact that we observed no dilatation of the skin vessels with this dose suggests that the fall in blood pressure is due to the dilatation of ves-

TABLE 3

The effect of amines given by stomach tube on gastric secretion

PROCEDURE	TIME	SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	3–4	0.7	0	0	
150 mgm. histamine at 4:00 per os in	4–5	3.8	25	55	
40 cc. water	5–6	9.5	87	107	
	6–7	2.5	52	80	
Control	3–4	2.0	0	10	
	4–5	3.5	65	95	
1 gram epinine at 4:00* per os in 40	5–6	12.0	117	137	Vomited at 4:30
cc. water	6–7	10.0	100	127	
	7–8	9.0	97	125	
	8–9	4.5	90	112	
Control	3–4	0.2	0	0	
	4–5	5.5	67	95	
1.5 grams ethylamine HCl at 4:00	5–6	4 0	50	82	
per os in 30 cc. water	6–7	2.5	57	95	
Control	2–3	2.2	0	10	
1.5 grams methylamine HCl at 3:00	3–4	6.0	37	57	
per os in 30 cc. water	4–5	7.7	70	95	
	5–6	3.5	55	90	
Control	3–4	3.3	0	15	Coeliacectomized
1 cc. amylamine at 4:00 per os in 30	4–5	8.2	50	70	dog
cc. water	5–6	4.5	40	62	Alkaline reaction
	6–7	3.0	35	57	neutralized
	2–3	0.6	0	0	
1 cc. iso-amylamine at 3:30 per os in	3–4	2.2	0	20	
30 cc. water	4–5	2.4	52	70	Alkaline reaction
	5–6	2.2	55	57	neutralized

* Epinine—3:4 dihydroxyphenylethylmethylamine.

sels in other areas. This suggests the following question: Can histamine cause gastric secretion without lowering blood pressure, and, if it does, is the secretion due to a local dilatation in the gastrointestinal area? This question is now being investigated by us.

It is very interesting to note that after the intravenous injection of histamine, the subcutaneous injection of a known stimulating dose causes very little response. The dog shown in table 2 usually secreted from 6 to 8 cc. of gastric juice the first hour after injection. Lim (9) has observed this post-histamine diminution of the response of the gastric glands on successive subcutaneous injections, it sometimes being as great as 50 per cent. Our results show that when the first dose is given intravenously the diminution in the response is greater than when given subcutaneously, being from 75 to 80 per cent less.

Observations with histamine on a denervated pouch: In the course of our work we have given 150 mgm. of histamine by stomach tube to two dogs with a "denervated" Heidenhain pouch (as completely denervated as possible without severing blood vessels) and have observed a marked stimulation of the glands of the pouch, the pouch being denervated six and eight weeks respectively.

This means that histamine when given per os can only act in one of three ways, either by finding its way into the circulation very slowly as occurs when injected subcutaneously or injected intravenously at a very slow rate, or by being changed on absorption by the intestinal mucosa and the new non-toxic substance acting as the gastric secretory excitant, or by a local action on the blood vessels of the gastric and intestinal mucosa thereby indirectly increasing the active circulation through the glands of the pouch.

Intestinal absorption and detoxication of histamine. No direct or conclusive evidence is available showing that histamine is actually absorbed from the intestine and passed into the blood as such.

Oehme (10) has given as much as 0.5 gram to rabbits by mouth without symptoms. We have given even 0.75 gram to normal dogs and 225 mgm. to man without symptoms (sometimes vomiting occurs) and Koessler and Hanke have given large doses to puppies, guinea pigs and dogs without symptoms of intoxication.

Popielski (11) found that the injection of histamine into the mesenteric veins causes the usual results. Meakins and Harington (12) have reported that histamine is absorbed from all parts of the intestinal tract and that the liver protected the general circulation from the absorbed histamine. Before the work of these latter investigators was published we had given from 0.5 to 0.75 gram of histamine by stomach tube to nine Eck fistula (chronic) dogs and found that such animals are as tolerant as normal dogs. This convinced us that the liver does not play an important rôle, if any, in protecting the general circulation from absorbed histamine.

This being the case histamine must either be absorbed at a very slow rate by the intestine or be changed by passage through the intestinal

mucosa. Since both Meakins and Harington and Koessler and Hanke found that histamine disappeared quite rapidly from the lumen of the intestine, and its disappearance is not due to change of the histamine in the lumen itself, histamine is apparently not absorbed at a slow rate. The latter investigators found no histamine in the portal blood following oral administration of large doses, but a small quantity was found in the liver and intestinal mucosa of dogs. Over 50 per cent of the histamine administered could not be accounted for. These data along with our observations on Eck fistula dogs practically prove that the intestinal mucosa either destroys or renders inert large quantities of histamine.

Although it is apparently true that histamine is changed by the intestinal mucosa, it is still possible that on administration of a large dose a quantity sufficient to stimulate gastric secretion (0.0027 mgm. per kilo per minute) passes through the intestine and even survives passage through the liver. That some does get to the liver of the dog is shown by the findings of Koessler and Hanke.

Summarizing, it is quite evident that histamine stimulates gastric secretion when administered subcutaneously, intravenously and by stomach tube provided the dosage is correct.

Epinine: 3:4 dihydroxyphenylethylmethylamine (Burroughs and Welcome) which has some of the pharmacologic properties of adrenalin, stimulates gastric secretion when given by stomach tube in doses of from 0.15 to 1 gram (tables 1 and 3). The larger dose (1 gram) not infrequently causes vomiting.

The subcutaneous injection of from 30 to 150 mgm. of epinine stimulates the gastric glands. Its latent period of action is from 40 to 60 minutes. We have not injected it intravenously.

Ivy and McIlvain (4) found that epinine caused the gastric glands to secrete when applied to the intestine. We have observed that stimulation occurs when 50 mgm. are given to dogs with a duodeno-esophageal anastomosis and a pouch of the entire stomach which confirms the findings of the above observers.

Epinine differs from adrenalin in that it does not have the alipathic hydroxyl group. When injected intravenously it causes a more prolonged rise in blood pressure, but not so intense, than adrenalin (14).

Ethylamine HCl: Ethylamine HCl was used because it was one of the possible decomposition products of β-alanine and some of the amino acids.

We have found that when from 0.5 to 1.5 grams of this substance is given by stomach tube the gastric glands are definitely stimulated (table 3).

Koch, Luckhardt and Keeton (13) injected subcutaneously 10 mgm. of tri and tetra ethylamine HCl with negative results. We have found

that as much as from 0.2 to 0.3 gram of ethylamine HCl can be injected subcutaneously without stimulating the gastric glands.

Methylamine HCl: What has been said concerning ethylamine HCl can also be said of methylamine HCl with the exception that it is a stronger excitant than ethylamine HCl (table 3).

Methylamine is the aporrhegma of glycine.

Ethylmethylamine HCl: Ethylmethylamine HCl when given by stomach tube in doses of from 0.4 to 1.0 gram is a stronger excitant than either ethyl or methylamine HCl (table 4). The latent period of stimulation is from forty to sixty minutes.

We have also observed that when 0.3 gram is injected subcutaneously stimulation occurs (table 9). The latent period of stimulation is the same as when it is administered by mouth.

n-Amylamine: n-Amylamine when given by stomach tube in from 1 to 2 cc. doses (alkalinity neutralized with HCl) excites a secretion of gastric juice (table 3).

We did not inject this substance subcutaneously as it is too irritating.

iso-Amylamine: iso-Amylamine per os stimulates gastric secretion but not to the degree that n-Amylamine does. We did not inject this substance subcutaneously.

Amylamine and iso-amylamine have a sympathomimetic action (15), (14). Amylamine is the aporrhegma of leucine and iso-amylamine is one of the pressor principles of putrid meat (17).

Hydroxylamine HCl: Hydroxylamine HCl when given by stomach tube (0.25–1 gram) and subcutaneously (0.2 gram) does not stimulate the gastric glands. Instead, it definitely depresses their activity and in most of our experiments completely inhibited the secretion.

This substance is said to be very toxic when given intravenously (14), but no symptoms were observed in our animals when given in the above manner.

Phenylhydroxylamine: Phenylhydroxylamine is very toxic when given by mouth. Less than 1 gram per os kills 10 kilogram dogs within thirty minutes of the symptom complex of asphyxia. The blood is chocolate brown in color and gives the spectrum of met-hemoglobin.

Phenylenediamine: Phenylenediamine when given per os in doses of from 200 to 500 mgm. does not excite the gastric glands. As is quite well known, this substance is toxic (15). Two 10-kilo dogs to which 500 mgm. were given developed general anasarca and died 48 hours later.

Subcutaneously 10 mgm. have no effect on the gastric glands and did not cause any objective phenomena. The para form was used.

iso-Propylamine: iso-Propylamine when given by mouth in doses of from 0.3 to 1.0 cc. in water (table 4) stimulates gastric secretion. Subcutaneously in 0.1 cc. doses in 5 cc. of water it is quite irritating and has no effect on the gastric glands.

Pyrrolidine: Pyrrolidine, the aporrhegma of proline, when given by stomach in doses of 1 cc. in 30 cc. of water stimulates gastric secretion. Subcutaneously it does not affect the gastric glands.

Pyrrolidine causes vaso-constriction (14), (18), (19), being the pressor principle of nicotine.

Glucosamine: As much as 3 grams of glucosamine by stomach tube had no effect on gastric secretion. Subcutaneously (300 mgm.) it is inert.

TABLE 4

The effect of amine given by stomach tube on gastric secretion

PROCEDURE	TIME	SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	2–3	6.0	67	102	
Control	3–4	6.2	65	95	Inhibition
1 gram hydroxylamine at 4:00 per os	4–5	2.5	50	85	
in 20 cc. water	5–6	1.3	0	15	
Control	3–4	3.0	0	12	
	4–5	15.0	82	100	Heidenhain pouch
1 cc. pyrolidine at 4:00 per os in 30	5–6	7.0	80	90	
cc. water	6–7	2.0	20	37	
Control	4–5	2.0	0	12	
30 cc. 1:5,000 adrenalin at 5:00 per	5–6	0	0	0	
os	6–7	3.5	37	50	Vomited at 6:15
Control	7–8	1.3	0	7	
1 cc. iso-propylamine in 20 cc. water	8–9	4.6	27	47	
per os at 8:00	9–10	4.6	30	52	
	10–11	4.5	0	15	
Control	1–2	1.1	42	47	Duodeno-esoph-
	2–3	2.5	37	50	ageal anasto-
0.75 gram ethylmethylamine HCl in	3–4	24.0	85	100	mosis and pouch of
20 cc. water at 2:00 into intestine	4–5	34.0	87	97	entire stomach
	5–6	23.0	67	97	

Koch, Luckhardt and Keeton (13) found that 20 mgm. did not effect the gastric glands when injected subcutaneously.

Tyramine: Tyramine phosphate when given by stomach tube in doses of from 0.15 to 1.0 gram,—the gastric glands were not stimulated. The two doubtful results consisted of a very slight rise in both the quantity and acidity of the secretion.

We have injected tyramine subcutaneously in doses of from 10 to 70

mgm. with negative results. Keeton, Koch and Luckhardt observed that 5 mgm. when injected intramuscularly inconstantly caused secretion in cats, but failed in all other animals.

Adrenalin: The literature on the effect of adrenalin on gastric secretion is conflicting. Yukawa (21) has reported that adrenalin given intravenously (8 drops in 1 cc. of water) and by mouth (8 drops in a capsule with a test meal) stimulates gastric secretion in most instances. Bonche (22) found that adrenalin (20 drops 5 minutes after a test meal) stimulates when given by mouth. Loeper and Verpy (23) have reported that the intramuscular injection of 1 mgm. of adrenalin in man augments the free and total acidity of the gastric contents. Rogers, Rahe and Ablahadian (24) observed an inhibition following the subcutaneous injection (3 cc. of adrenalin). Boenheim (25) has made observations on man which he states confirm the observations of Rogers, et al. Hess and Gundlach (26) found that when adrenalin was given intramuscularly (0.5 to 10 cc. of 1:1000) or intravenously (0.5 cc of 1:1000) gastric secretion was depressed. Rothlin (27) reports that it is negative intravenously and intramuscularly. Lim (7) observed that intravenously (1 cc. of 1:10,000) in cats it sometimes stimulates. Ivy and McIlvain (4) found that when a 1:50,000 solution of adrenalin was applied to a Thiry's fistula of the intestine gastric secretion was stimulated. Hernando (28) making observations on patients observed that adrenalin by mouth (10 drops 1:1000 three times a day) augments gastric secretion; intramuscularly (0.25 mgm.) diminishes it, and intravenously (0.1 to 0.05 mgm.) diminishes or has no effect. In a case of pyloric stenosis augmentation was observed when 0.25 mgm. was injected intravenously. Bennett (29) has reported that adrenalin subcutaneously and by mouth (dose not ascertainable) has no effect on gastric secretion.

Per os: We have given adrenalin by stomach tube in doses of from 30 to 50 cc. of from 1:3,000 to 1:50,000 without observing a single instance of definite stimulation. A result that might be termed slightly positive is shown in table 4. (With strong solutions vomiting frequently occurs.) This finding was quite surprising in view of the observations of Ivy and McIlvain (4). The discrepancy can be explained, however, by assuming that the adrenalin is destroyed by the pancreatic secretion present in the intestine, but not in a Thiry's fistula.

Subcutaneously: Subcutaneously and intramuscularly (5 experiments each) we have found that from 0.5 to 1.0 cc. of 1:1000 adrenalin either slightly depresses or has no effect on gastric secretion.

Intravenously: We have used various dilutions and rates of injection; 1:500,000 and 1:1 million adrenalin was injected intravenously at a rate of 0.5 cc. to 1.0 cc. per minute, respectively, for 25 minutes in four experiments on two dogs, one weighing 10 kilos, the other 12, without stimulating

gastric secretion. One of the dogs vomited one hour after the injection and refused to eat later. The other animal was unaffected. On the basis of Lim's and Hernando's observations the dose was increased. Dosages used were as follows: 1 to 2 cc. of 1:1000 (4 experiments); 1 to 2 cc. of 1:25,000 (2 experiments each); 1 to 12 cc. of 1:100,000 (4 experiments); 10 to 12 cc. of 1:200,000 (10 experiments). The smaller quantities of solutions were injected within 15 seconds and the larger quantities (10 cc.) in from 90 to 120 seconds. The animals used weighed from 10 to 12 kilos. These injections resulted in only two positive results and they occurred in the same dog on the same day (table 2). Four attempts to repeat this observation on the same dog on different days failed.

We are forced to the same conclusion arrived at by Lim (7), namely, that adrenalin intravenously sometimes stimulates.

According to Hoskins and Gunning (34) and Hartman and McPhedran (35) the last series of doses of adrenalin we used (1:100,000 and 1:200,000) causes dilatation of and increased blood flow through the blood vessels of the intestine lasting from 5 to 10 minutes in anesthetized animals. If we might assume that such a phenomenon occurs in the stomach of unanesthetized animals, there is at hand an explanation of the stimulation of gastric secretion when it occurs. The infrequency of the occurrence of stimulation is best accounted for by the briefness of the period of vasodilatation and increased blood flow. This explanation is supported by our observations on epinine which acts pharmacologically like adrenalin with the exception that its action is more prolonged.

OTHER SUBSTANCES. *Choline HCl: Per os.* From 0.5 to 1.5 gram of choline HCl (free acid neutralized) when given by stomach tube in 30 cc. of water stimulates gastric secretion markedly (tables 6 and 7). The latent period of stimulation in most cases was one hour.

The experiment shown in table 7 demonstrates that the site of action of choline given by mouth is in the intestine.

Subcutaneously: From 0.2 to 0.5 gram of choline HCl subcutaneously stimulates the gastric glands; 0.2 gram does not always stimulate, but 0.5 gram has never failed. The latent period is usually within thirty minutes. In this respect it is similar to gastrin.

Our doses are larger than those used by Koch, Luckhardt and Keeton (13) who injected from 10 to 20 mgm. subcutaneously and observed five positive results, ten negative and one doubtful. They conclude from their observation with the dose they used that "choline acts with uncertainty and then only faintly as compared with histamine or gastrin." Chiari (28) has reported that choline acts on the gastric glands like pilocarpine, but does not give the dose used. Von Fürth and Schwarz (25) found that choline (1 to 3 mgm.) stimulated the salivary glands and pancreas, but expressed the belief that it was not the active principle of pancreatic secretion.

Choline is present in the intestinal mucosa (25), (26) and its action is antagonized by atropin. The questions that remain to be answered are: whether there is sufficient choline in gastrin to account for its action? If not, may small amounts of choline act synergistically with some other substance in gastrin? And how antagonistic are choline and atropin when acting on the gastric glands? These questions we hope to answer.

A small dog (8 kilos) that received 0.5 gram of choline HCl subcutaneously salivated and lacrimated copiously.

Betaine HCl: Trimethylglycine HCl was given by stomach tube in doses of 1 and 2 grams (free acid neutralized) with the result that gastric

TABLE 5

The effect of various substances administered by mouth on gastric secretion. Summary

Pavlov pouch dogs

SUBSTANCE	DOSE	NUMBER OF EXPERIMENTS	RESULTS		
			Positive	Negative	Doubtful
Choline HCl	0.5–1.5 grams	5	4	1	0
Betaine HCl	1–2 grams	10	6	3	1
Sarcosine	2 grams	4	3	0	1
Malonic acid	1.5 grams	4	0	3	1
Pyruvic acid	1–2.5 grams	4	0	3	1
Hydracrylic acid	0.3–1 gram	14	7	5	2
Methyl bichloride	3 cc.	5	3	1	1
Guanidine HCl	0.5 gram	3	0	2	1
Chloroform	3 cc.	3	3	0	0
Ether	3 cc.	3	2	0	1
-chlor proprionic acid	0.5 gram	6	6	0	0
Skatol	0.5 gram	3	3	0	0
Mineral oil	250 cc.	10	1	9	0
Croton oil	1–3 drops	3	0	3	0
Picric acid	0.4 gram	3	3	0	0

secretion was frequently stimulated (table 5). Subcutaneously it is negative (300 mgm.).

Sarcosine: Sarcosine (Eastman) when given by stomach tube in 2 gram doses stimulates gastric secretion slightly (table 6).

Komaroff (27) reports that carnosine stimulates gastric secretion when given subcutaneously and intravenously. Carnitine also stimulates, but is less active.

Malonic and pyruvic acids: Malonic and pyruvic acids were used because they are possible decomposition products of α and β alanine. These substances were negative.

Hydracrylic acid: β-lactic acid was used because it is a possible byproduct of β-alanine.

When from 0.3 to 1.0 cc. of hydracrylic acid is given by stomach tube (free acid neutralized) in 30 cc. of water definite stimulation results (table 7).

TABLE 6

Effect of various substances administered by mouth on gastric secretion

PROCEDURE	TIME	SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	3–4	1.8	0	10	
0.5 gram choline HCl at 4:00 per os in 20 cc. water	4–5	2.0	0	12	
	5–6	6.0	62	82	
	6–7	8.7	85	100	
1 gram choline HCl at 5:15. See table 7	7·8	5.7	62	87	
	8–9	5.0	45	75	
Control	2–3	1.9	25	37	
1 gram betaine HCl at 3:00 per os in 20 cc. water	3–4	4.5	65	77	
	4–5	4.0	65	82	
	5–6	4.5	85	97	
Control	2–3	1.0	0	7	
2 grams sarcosine at 3:00 per os in 30 cc. water	3–4	3.1	22	37	
	4–5	1.0	22	40	
Control	3–4	1.2	0	0	
1.5 grams malonic acid at 4:00 per os in 30 cc. water	4–5	1.8	12	37	Doubtful
	5–6	1.5	10	35	
Control	2–3	0.2	0	0	
0.5 gram skatol in 20 cc. water per os at 3:00	3–4	0.8	0	17	
	4–5	3.1	75	110	
Control	2–3	2.0	25	32	
250 cc. mineral oil at 3:35 per os	3–4	2.1	17	35	
	4–5	4.0	75	87	
	5–6	2.0	70	85	
	1–2	2.0	0	27	
0.5 gram β-chlor propionic acid per os in 20 cc. water at 2:30	2–3	2.8	0	47	
	3–4	7.5	87	105	
	4–5	2.0	40	85	

Guanidine HCl: We have found that guanidine is negative when given subcutaneously or by stomach tube. Half a gram by stomach tube sometimes caused vomiting.

Koch, Luckhardt and Keeton (13) found that it was negative subcutaneously (10 mgm.).

Komaroff (27) reports that methyl guanidine is slightly positive.

Methylbichloride, chloroform and ether: When 3 cc. of methylbichloride is given by stomach tube the gastric glands are stimulated slightly. The same is true of chloroform and ether.

TABLE 7

The effect of various substances administered by mouth on gastric secretion

PROCEDURE	TIME	SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	1–2	1.3	0	5	
3 cc. methyl bichloride per os with 20 cc. water at 2:30	2–3	2.0	25	45	
	3–4	2.4	62	80	Vomited at 3:10
	4–5	1.0	52	77	
Control	9–10	2.5	52	70	
Control	10–11	3.5	50	62	
1 cc. hydracrylic acid in 20 cc. water at 11:00	11–12	8.0	75	97	
	12–1	4.3	70	87	
	1–2	1.5	27	50	
Control	1–2	3.0	0	7	
3 cc. chloroform at 2:00 with 20 cc. water	2–3	5.0	30	55	
	3–4	4.5	60	80	
	4–5	2.5	60	77	
Control	11–12	1.2	0	10	
3 cc. ether at 12:00 in 40 cc. water per os	12–1	2.5	15	32	
	1–2	1.2	7	40	
	2–3	1.5	0	17	
Control	2–3	1.7	10	25	
0.5 gram picric acid at 3:00 in 40 cc. water per os	3–4	2.6	62	72	
	4–5	5.0	95	105	
	5–6	1.0	80	87	
Control	11–12	0.2	0	5	Dog: "Benj."
Control	12–1	1.0	0	5	Pouch of entire stomach and duodeno-esophageal anastomosis
Control	1–2	1.0	0	5	
20 cc. 2 per cent choline HCl applied to stomach 2:10–2:35	2–3	1.0	0	5	
	3–3:30	10.0	35	50	
20 cc. returned	3:30–4	18.0	85	102	
40 cc. 2 per cent choline at 3:00 introduced into intestine	4–4:30	25.0	102	130	
	4:30–5	12.0	80	117	

This stimulation is not due to distention because in these experiments the stomach tube was held in place for three minutes after the administration of the fluid (tables 5, 6 and 7).

Skatol: Skatol in 0.5 gram doses in water by stomach tube stimulated the gastric glands (table 6).

Mineral oil and croton oil: Mineral oil (250 cc.) plus 50 grams "cellu" flour stimulated gastric secretion in one experiment out of ten (table 6). In the other experiments depression of secretion occurred. The catharsis was marked and occurred within an hour.

Croton oil was negative in three experiments. Catharsis did not occur to the extent that it did when mineral oil was used.

Picric acid: Picric acid stimulates both subcutaneously and by stomach tube. This was discovered when lysine picrate was used in our previous article.

The gastric glands when stimulated by picric acid continue to function even while the animal is undergoing violent convulsions.

TABLE 8

Effect of the subcutaneous injection of amines and other substances on gastric secretion. Summary

SUBSTANCE	DOSE	NUMBER OF EXPERIMENTS	RESULTS		
			Positive	Negative	Doubtful
	gram				
Epinine	0.035–0.15	8	7	1	0
Tyramine	0.010–0.070	13	0	11	2
Choline HCl	0.2–0.5	7	6	1	0
Picric acid	0.4	2	2	0	0
Ethylmethylamine HCl	0.2–0.3	6	6	0	0
Guanidine hydrochloric	0.2–0.5	3	0	3	0

β-chlor propionic acid: β-chlor propionic acid (free acid neutralized) stimulates gastric secretion when given (0.5 gram) by mouth in 20 cc. of water. Its latent period of stimulation is from thirty to sixty minutes. This substance was used because it is one of the sources of β-lactic acid.

DISCUSSION. Of the fourteen amines used all stimulate gastric secretion except four, when acting in the intestine. The dose required is relatively large.

These observations probably have definite physiological and pathological significance. But before such a conclusion can be made we must know more concerning the rate of production and concentration of amines in the intestine and factors that are concerned in their absorption.

With reference to the mechanism by which the amines excite gastric secretion, we directed most of our attention to histamine, under which topic we have discussed our findings.

Of the possible derived products of β-alanine, ethylamine and β-lactic acid are the only ones that stimulate gastric secretion when given by stom-

ach tube. Since neither of these substances is more potent than β-alanine itself, it seems quite likely that β-alanine acts per se.

Since methyl bichloride, chloroform and ether belong to the same general pharmacologic group as alcohol, it was expected that they would stimulate gastric secretion on administration by stomach tube (4).

That drastic catharsis usually depresses the continuous secretion of gastric juice strongly suggests that the secretion is due to amines or other substances acting via the intestine. This observation is quite similar

TABLE 9

The effect of subcutaneous injection of various substances

Pavlov pouch dogs

PROCEDURE	TIME	SECRETION			REMARKS
		Amount	Free acid	Total acid	
	o'clock	*cc.*			
Control	10–11	1.2	37	57	
	11–12	1.0	0	12	
30 mgm. epinine at 11:00 subcutaneously	12–1	5.9	80	87	
	1–2	2.0	77	90	
	2–3	1.0	20	30	
	3–4	1.8	0	10	
Control	3–4	5.0	0	12	
0.5 gram choline acid at 5:00 subcutaneously	4–5	45.0	80	100	Salivation
	5–6	10.0	80	100	
	6–7	5.0	15	47	
Control	4–5	0.1	0	0	
0.4 gram picric acid at 5:00 subcutaneously	5–6	4.0	82	105	
	6–7	2.9	92	107	
Control	1–2	0.5	20	25	
	2–3	2.5	45	62	
0.3 gram subcutaneously of ethylmethylamine HCl at 2:00	3–4	5.4	85	97	
	4–5	5.0	87	100	
	5–6	3.0	87	0	

to the results reported (paper IV) on the use of hydrolyzed proteins, namely, that if they passed through the gastro-intestinal tract rapidly, no stimulation of the gastric glands resulted. Dehydration would be an additional factor depressing secretion in active catharsis, but this did not operate to any extent in our experiments with mineral oil and "cellu" flour.

CONCLUSIONS

1. Histamine, "epinine," ethylamine HCl, methylamine HCl, ethylmethylamine HCl, iso-amylamine, amylamine, pyrolidine, iso-propyl-

amine, choline, betaine HCl, sarcosine, β-lactic acid, β-chlorpropionic acid, picric acid, methyl bichloride, chloroform, ether and skatol in certain doses by stomach tube stimulate the gastric glands.

2. Histamine, "epinine," ethylmethylamine HCl, choline and picric acid in certain doses subcutaneously stimulate the gastric glands.

3. Histamine intravenously in a dose of 0.0027 mgm. per kilo per minute stimulates the gastric glands. Adrenalin sometimes slightly stimulates when given intravenously.

4. Histamine stimulation leaves the gastric secretory mechanism refractory, the degree depending upon the mode of administration.

5. The normal intestinal mucosa possesses the power of rendering inert relatively large quantities of histamine.

6. Glucosamine, tyramine, adrenalin, malonic acid and pyruvic acids, and guanidine HCl do not stimulate the gastric glands when given by mouth or subcutaneously. Adrenalin, however, sometimes causes slight stimulation when given by mouth.

7. Adrenalin subcutaneously or intramuscularly sometimes inhibits gastric secretion. Hydroxylamine HCl given by mouth inhibits gastric secretion.

8. Phenylhydroxylamine is a very toxic substance.

9. Choline HCl in certain doses subcutaneously has a latent period of stimulation not unlike that of gastrin. When given by mouth it acts via the intestine and not via the stomach.

10. Drastic purgation of the gastro-intestinal tract usually depresses and only rarely leads to increased secretion of gastric juice.

BIBLIOGRAPHY

(1) Koskowski: Compt. Rend., 1922, clxxiv, 247.
(2) Popielski: Pflüger's Arch., 1920, clxxviii, 214.
(3) Rothlin and Gundlach: Arch. Internat. d. Physiol., 1921, xviii, 59.
(4) Ivy and McIlvain: This Journal, 1923, lxvii, 124.
(5) Ivy, McIlvain and Javois: Science, 1923, lviii, 286.
(6) Lim, Ivy and McCarthy: Quart. Journ. Exper. Physiol., 1925, xv.
(7) Lim: Quart. Journ. Exper. Physiol., 1922, xiii, 79.
(8) Koessler and Hanke: Journ. Biol. Chem., 1924, lix, 889.
(9) Lim: This Journal, 1924, lxix, 322.
(10) Oehme: Arch. exper. Path. et Pharm., 1913, lxxii, 76.
(11) Popielski: Pflüger's Arch., 1920, clxxviii, 237.
(12) Meakins and Harington: Journ. Pharm. Exper. Therap., 1923, xx, 45.
(13) Koch, Luckhardt and Keeton: This Journal, 1920, lii, 508.
(14) May: The chemistry of synthetic drugs. London, 1911.
(15) Sollman: A manual of pharmacology. Philadelphia, 1922.
(16) Barger and Dale: Journ. Physiol., 1910, xlii, 19.
(17) Barger: The simpler natural bases. London, 1914.
(18) Tunnicliffe and Rosenheim: Centralbl. f. Physiol., 1902, xvi, 93.
(19) Pictet and Court: Ber. deutsch. chem. Gesellsch., 1907, xl, 3771.

(20) KEETON, KOCH AND LUCKHARDT: This Journal, 1920, li, 455.
(21) YUKAWA: Arch. f. Verdauungskrankheiten, 1908, xiv, 166.
(22) BONCHE: Diss., Freiburg, 1909, loc. cit. Boenheim.
(23) LOEPER AND VERPY: Soc. de Biol., 1917, lxxx, 703.
(24) ROGERS, RAHE AND ABLAHADIAN: This Journal, 1919, xlviii, 79.
(25) BOENHEIM: Arch. f. Verdauungskrank., 1920, xxvi, 74; Deutsch. Med. Wochenschr., 1921, xlvii, 1256.
(26) HESS AND GUNDLACH: Pflüger's Arch., 1920, clxxxv, 121.
(27) ROTHLIN: Communication to Int. Physiol. long., Paris, 1920.
(28) HERNANDO: Presse Med., 1923, xxxi, 797.
(29) BENNETT: Proc. Roy. Soc. Med. (Therap.), 1923, xvi, no. 3.
(30) VON FÜRTH AND SCHWARZ: Pflüger's Arch., 1908, cxxiv, 427.
(31) LE HEUX: Pflüger's Arch., 1918, clxxiii, 8; 1920, clxxix, 177.
(32) KOMAROFF: Physiol. Abst., 1924, viii, Abst. no. 364.
(33) CHIARI: Ther. Monat., 1915, xxix, 202.
(34) HOSKINS AND GUNNING: This Journal, 1917, xliii, 298, 399.
(35) HARTMAN AND MCPHEDRAN: This Journal, 1917, xliii, 311.

Note: We desire to thank Messrs. H. E. Schmitz and G. B. Fauley for the assistance they have given us in part of this work.

THE CAUSE OF MANY FEBRILE REACTIONS FOLLOWING INTRAVENOUS INJECTIONS. I

FLORENCE B. SEIBERT[1]

From the Department of Pathology of the University of Chicago

Received for publication October 2, 1924

A knowledge of the causes of the physiological reactions produced in the body by the intravenous injection of water is essential for an understanding of the effects following injections of any aqueous solutions. Even with solutions made isotonic with the blood, chill and fever reactions often occur, and these reactions have been misinterpreted in many cases. Intravenous injections of saline, dextrose, salvarsan, neo-salvarsan, numerous salt and protein solutions should not be followed by chill and fever, and it is the purpose of this paper to explain how such reactions may be avoided.

It is part of a modern physician's training to realize the necessity of using freshly distilled water for intravenous injections, but a knowledge of why this is necessary will eliminate some causes of error still present. That there is a measurable fever-producing substance in some distilled waters has been proved in a previous paper (1), and the reactions produced by injecting these waters are due to the effect of this substance upon the body, rather than to any physical change produced in the blood by the presence of water. It was shown that this substance can be successfully eliminated only by distilling the water through an apparatus containing a spray-catching trap. Distilled water, if properly purified, can be injected in extraordinarily large amounts into rabbits without any effect upon temperature.

The substance was shown to be a product formed by bacteria which contaminate the water, to retain its fever-producing power even after filtration through the Berkefeld, to be very resistant to heat, and to develop in water in quantity sufficient to produce fever, in four days at most from the time the water was contaminated with the organism.

Furthermore, it was demonstrated (2) that these facts apply to protein solutions as well as to water for, by eliminating bacterial contamination during the preparation of casein, it was possible to make this protein so that no reaction followed its intravenous injection. On the other hand,

[1] The research was carried on under tenure of the Porter Fellowship, given by the American Physiological Society, 1923–24.

casein prepared in the usual manner always gave rise to fever. Preparations of three other proteins, edestin, ovalbumin and hemoglobin, all gave results suggestive, although not absolutely constant, of the theory that the pyrogen is a bacterial contaminant rather than the protein under observation.

A more detailed study of the properties of this pyrogen, of its prevalence, and of the specific organisms producing it, is necessary as a foundation for work in this field. It was for this reason that the present investigation has been undertaken. A review of the literature on these subjects is given in the two papers referred to.

EXPERIMENTAL. Rabbits were used in most of the experiments in these studies and what has been found to be true in regard to fever production in these animals applies also to man. After reviewing the data obtained in experiments upon 110 rabbits, 40 of which were reported in a previous paper (1), there is no doubt as to the reliability of the fever test upon rabbits. All combinations of experiments conceivable upon the same rabbit at different times or upon different rabbits at the same time produced consistent and comparable temperature fluctuations, as long as the preliminary temperatures of the rabbits before the experiment were within the normal range. For example, freshly distilled water, even in 15 cc. doses, never produced fever in any rabbit regardless of the number of previous fevers the same rabbit had displayed.

In the present study 37 such samples of water were tested, making a total of 90 with those reported previously. Similarly a water which once produced a fever could be shown to do so at any time and in any number of rabbits. The record of each animal was kept, but such protocols are too extensive for publication, some animals having received as many as 75 injections of 5 to 15 cc. amounts.

Waters from various sources were studied and it was found that the same general statements could be made concerning the fever-producing properties of waters obtained in Chicago as were made in regard to those obtained the previous year in New Haven, Conn. (1). Tap water on three different occasions, distilled waters taken from four different stock containers, and three separate lots of commercial "Neptune" distilled water, obtained at different times, all produced fever when injected intravenously into rabbits in 5 cc. doses.

That the pyrogenic substance found in these waters is due to a product formed by bacteria which contaminate the waters is supported by much further evidence. By way of summary, reference will be made to facts accumulated in a former paper (1), many of which have been further confirmed. For example, it was shown that fever is not caused by the manipulation of the animals necessary to the determination of hourly temperatures. It is not dependent upon the rate of injection of the water

and it is not due to inorganic impurities in the water. Furthermore, the resistance and hypersensitiveness of the individual animals and the influence of previous injections do not determine the fever, nor does hemolysis of the blood and hydrogen ion concentration of the solutions injected.

Bacteriological nature of the pyrogen. The best evidence for the statement that the pyrogen is a product formed by bacteria which contaminate the water is contained in the following facts. Non-fever-producing waters may spontaneously become fever-producing after standing at least four days under non-sterile conditions, and bacteria can be isolated from such waters. The bacterial nature of the pyrogen was established by the following experiment.

A sample of freshly distilled water, which was shown to have no pyrogenic effect was divided into three parts and each part sterilized. Part 1 was then inoculated

TABLE 1

	TESTED IMMEDIATELY		TESTED AFTER ONE WEEK	TESTED AFTER THREE MONTHS	
	Bacteria per cubic centimeter	Fever	Fever	Bacteria per cubic centimeter	Fever
	colonies	*°C.*	*°C.*	*colonies*	*°C.*
Part 1. Water inoculated with 0.1 cc. fever-producing water −41	5	0.15	1.1	1450	1.7 1.85
Part 2. Water inoculated with 0.1 cc. Berkefeld filtrate of water 41	0	0.05	0.0	0	0.0 0.05
Part 3. Sterile water untreated	0	0.05 0.05	0.2	0	0.3 0.15

with 0.1 cc. of a fever-producing water. Part 2 was inoculated with 0.1 cc. of a fever-producing sterile Berkefeld filtrate of this water. Part 3 was left untouched and kept sterile. All three samples tested immediately did not produce fever, and all were shown to be sterile except 1, which contained 5 colonies per cc. After standing a week they were again tested. Part 1 produced a fever, indicating multiplication of the bacteria and development of pyrogen. Part 2 produced no fever, indicating that a filterable virus, which can not be detected on the bacteriological plate, is not the means by which pyrogen develops. Part 3, the control, produced no fever. These were again tested after standing three months and the results obtained were in the same order, as seen from table 1.

Therefore, the presence of bacteria is necessary for the development of pyrogen in distilled water, since freshly distilled water may be kept for months under identical conditions without producing fever, provided its sterility is preserved. Moreover, the pyrogen is a product of bacterial origin and is not due to a filterable virus, since inoculation with a fever-producing Berkefeld filtrate of the same water did not lead to development of pyrogen.

Advantage has been taken of these facts in making fever-producing waters on a large scale. As much as 12 liters of a sterile freshly distilled water have been rendered fever-producing by an inoculation with 4 cc. of a pyrogenic water. The different waters studied are listed in table 2 along with the fever produced by their injection. As explained in a previous paper (3), anything above 0.5°C. rise in temperature was considered a fever, the injections were all made intravenously and hourly rectal temperatures were taken. In the following table 2 are given the average rises in temperature following the injection of 5 cc. amounts of the waters. Since the height of the temperature curve cannot be increased with larger doses after the maximum is once reached, there is also recorded—column 3—the minimum amount of water which will just produce a fever. This is a much more accurate means of measuring the potency of a water.

Since it is therefore evident that certain distilled waters when injected into rabbits produce fever because they have been contaminated with bacteria, we must look to the bacteria for material for studying the nature of the pyrogen. A study of the bacteriology of many of the fever-producing waters has been made and will be presented in Part II. These studies, however, indicate that the fever is caused not so much by numbers of bacteria as by types and this will be shown to be the case later when the relationship between groups of bacteria and physiological reaction produced by them is discussed.

For example, water 2a which did not produce fever was shown to contain at first so many bacteria that they could not be counted in 0.1 cc. dilution. About a month later the count of living bacteria reduced to 685 per cubic centimeter and still the water did not produce fever. On the other hand, water 36 showed a continual decrease in viable bacteria from 475 per cubic centimeter to none per cubic centimeter while it was being incubated at 37°C., and still continued to be one of the most pyrogenic waters. These results in this last experiment are of further interest because they indicate that the pyrogen is a product formed by bacteria and cannot necessarily be measured by a count of living bacteria.

As to the nature of the pyrogen, it is a soluble, filterable product of bacterial origin. It is not essential for the pyrogen to be in the form of intact bacteria in order to provoke the fever reaction, since Berkefeld filtrates of potent waters elicit fever as well as the unfiltered solutions. The pyrogen is, therefore, a chemical substance in solution or a colloidal aggregate of a size which will penetrate the pores of the porcelain candle. As noted before, it is not a living substance of the nature of a virus or of bacteria undetectable by the plate method, since inoculation of non-fever-producing waters with Berkefeld filtrates of fever-producing waters did not lead to development of the pyrogen, as did occur following inoculation with unfiltered water containing recognizable organisms.

If distilled waters contain a pyrogenic substance, because they are contaminated with bacterial products, then it should be possible to make such products from pure cultures of the various types of bacteria and so detect whether one or more than one type is responsible for the pyrogen in the water. This has been done, and it is evident that the pyrogen is a product of specific strains of bacteria and is not produced by all bacteria which may be present. The bacterial flora of twelve different distilled waters were studied and of the organisms isolated about thirty strains were tested for fever-producing material. Of all the waters studied, certain ones naturally have the same flora since they were inoculated from the same water. For

TABLE 2

Fevers produced by various waters

NUMBER OF WATER	AVERAGE RISE IN TEMPERATURE WITH 5 CC. AMOUNTS	AMOUNT WATER REQUIRED FOR MINIMAL FEVER
	°C.	cc.
8	1.35	
10	0.94	5
21	2.00	
2 a	0.30	
3	0.85	
23 a	1.20	
12	1.20	1/5
31	1.60	1/2
	1.80	
36 (20°C.)	1.30	1/2
	1.30	1/2
36 (37°C.)	1.70	1/2
	1.35	
33	1.35	1/10
41	1.20	1/10
12-1	1.10	3
12-2	1.10	1
30	0.30	
Sterile water	0.00	

example, waters 10, 20, 21 and 23B were freshly distilled waters which were inoculated with water 8, which in turn was a water that had become pyrogenic spontaneously. Similarly, waters 12, 31, 36 (20°C.), 36 (37°C.), 41 and 42 belong in the same group, all having been inoculated with water 12. Water 23B received an inoculation from both 10 and 12. Waters 12, 12-1 and 12-2 were three lots of a commercial distilled water ("Neptune") purchased at three different times. Waters 2a and 3 were distilled waters taken from different containers about the laboratory. Water 30 was a freshly distilled water kept under sterile precautions.

These waters were plated on plain nutrient agar and an attempt was

made to isolate all the types of bacteria in any water or in any group of waters, which gave promise of being different, because of some peculiar colony characteristics. Doubtless some types of organisms were overlooked because of the similarity of their colonies to colonies already selected.

The common bacteriological tests in regard to cultural growths and reactions were made upon the strains isolated and this study in detail, will be given in Part II of this paper. It was found that all the bacteria were slow growers and could be grouped as chromogenic and non-chromogenic organisms and certain outstanding characteristics were common to all of them. In the first place, all survived in distilled water and many for months, presumably upon the gases absorbed from the air or upon their autolyzed predecessors. They therefore seem to fall within the group of protrophic bacteria according to Prescott and Winslow (4). Further properties common to all of the bacteria studied were non-liquefaction of gelatin and the failure to produce acid and gas in dextrose, lactose and saccharose broths. However, there was one exception to this rule—A31/5—which produced acid in dextrose broth after seven days. All other properties and similarity to known organisms are discussed in Part II.

Physiological reactions of isolated strains of bacteria. At the same time that the cultural studies of the bacteria were being made, independently the physiological reactions in rabbits were tested, and the organisms were again grouped according to this property. It has been mentioned before that the intact bacteria are not required to produce fever in rabbits and that Berkefeld filtrates of pyrogenic waters are very potent. Therefore, it should be possible to obtain products for study from the various bacteria by filtering their water extracts through the Berkefeld. This was the method used for studying the physiological reactions of the various strains of bacteria.

Technic. The method of preparing the material for testing is as follows: One agar slant of a pure culture of the bacterium under investigation was suspended in 100 cc. of sterile freshly distilled water and incubated over night. The following morning the suspension was filtered through a sterile Berkefeld candle into a sterile flask and 5 cc. of this filtrate were then injected intravenously into rabbits in order to study the quantity of pyrogen present.

The controls which are of the greatest importance in an experiment of this nature seemed adequate. At first the freshly distilled water used for the suspension was always proved by a test to have no effect upon the temperature fluctuation when injected into rabbits. But after 90 such tests were made, all of which gave negative results, with never a single positive reaction, it seemed safe to assume that freshly distilled water, prepared as mentioned above and used while fresh, had no pyrogenic effect.

The filtration through the Berkefeld candle proved to be the most probable source of error. It was found that almost any amount of washing with soap and water and subsequent sterilization did not free the candle of pyrogenic material which upon filtration of the substance to be tested was carried through, thus rendering the filtrate fever-producing. This was shown to be the case on at least thirteen different occasions by filtering a non-fever-producing water through candles cleaned in the ordinary manner. The only reliable method which was discovered for cleaning Berkefeld candles satisfactorily is the following.

At first antiformin was used, but later it was found that 15 per cent NaOH, if allowed to stand over night in the Berkefeld cup and to filter through the candle at its own rate, was an adequate solvent for the bacteria. Then the candle was gently brushed under running water and about a liter of freshly distilled water was filtered with suction in order to wash away the alkali. Immediately the complete apparatus ready for a filtration, was autoclaved for fifteen minutes at fifteen pounds pressure.

Thirty-eight such candles were shown to be free from pyrogen when tested with freshly distilled water and only candles so tested were used in the following experiments. The life of the candle seemed to be shortened considerably by this drastic treatment and there was an attempt to determine whether merely filtering alkali through the candle several times followed by the usual washing, would suffice. It was found, however, that candles treated in this way were not free from pyrogen. This fact must therefore be considered when the fever-producing property of any Berkefeld filtrate is studied.

A third control consisted in proving that the water of condensation on an agar slant, as well as any minute pieces of agar accidentally scraped off with the bacteria for the suspension were not the course of pyrogen. One sterile agar slant was washed into about 100 cc. of freshly distilled water and incubated over night. Another whole slant was macerated in 100 cc. of freshly distilled water and autoclaved in order to put it into solution. These two solutions were then filtered through a Berkefeld candle the following day and 5 cc. portions of the filtrates produced no fever when injected into rabbits. In the latter cases the 5 cc. of solution contained 7.5 mgm. agar and 2.5 mgm. of meat extract, and these were consequently insufficient to produce fever. Therefore, the pyrogen is not a substance produced from or contained in the medium used for cultivating the bacteria.

Correlation of bacteriological and physiological groups of bacteria isolated. With such controls it seemed that the reactions produced in rabbits following the injections of these Berkefeld filtrates were due to products formed by or autolytic products of the bacteria under consideration.

TABLE 3

Correlation of bacteriological and physiological studies

BACTERIAL PROPERTIES	EXTRACT OF CULTURE	WATER SOURCE	GROUP REACTION	RISE IN TEMPERATURE	N PER 5 CC.	NUMBER OF DEATHS
Chromogenic bacteria						
				°C.	*mgm.*	
Yellow pigment	B36/5	36	I	0.3, 0.6, 0.0	0.0080	
	B12/3	12	I	0.0, 0.2	0.0040	
Pink pigment	D2a/1	2 a	I	0.0, 0.1	0.0040	
Motile Milk alk. Nitrates not red.	D2a/3	2 a	I?	1.1, 0.3	0.0050	1?
Non-motile Milk alk. Nitrates not red.	D2a/2	2 a	I	0.2, 0.3	0.0040	
Motile Milk alk. Nitrates reduced.	B10/7	10	I	0.0, 0.1	0.0044	
Gram +	OCI	New Haven water	I?	0.7	0.0060	
Non-chromogenic bacteria						
Motile Milk alk.	B12-2/6	12–2	II	1.7, 1.6	0.0128 0.0200	
Nitrates not red. or reduced.	A12-1/8	12–1	II	1.0 0.7	0.0200	
Non-motile Milk alk. Nitrates not reduced.	B12-2/4	12–2	II	1.8, 1.0	0.0032	
	A12-1/9	12–1	I	0.1, 0.0, 0.5	0.0048	
	B36R/4	36 (20°)	II	1.0, 0.9	0.0028	
	B12/8	12	II	0.8, 0.8	0.0008	
Sluggish	B10/8	10	III	1.1, 1.5	0.0028	
	B20/4	20	III	2.2, 2.0	0.0068	
Milk acid; coag. Nitrates reduced or not reduced.	B23B/4	23 before	III	1.7, 2.1	0.0048	
	B23B/1	23 before	III	1.5, 0.8	0.0040	
	B20/1	20	III	1.4, 1.5	0.0032	
	B23A/1	23 after	III	1.7, 1.4	0.0024	
	A31/1	31	IV	1.0, 2.0 1.5, 1.5	0.0108 0.0100	2 2 } 4
	A31/5	31	III or IV?	2.3 2.2	0.0024	
Non-motile Milk acid; pepton; and sec. alk. Nitrates not red.	B36/4	36 (37°)	IV	1.3	0.0006 0.0032	10
Non-motile Milk acid; coag.; peptonization Nitrates reduced.	OTI	New Haven water	III		0.0060	

		Special			
Gram +	OCIII	New Haven water	I	0.2	0.0050
Coccus Gram +	B8/6	8	I	0.3, 0.2	0.0040

Four groups of organisms can be formed on the basis of the physiological reactions produced in rabbits.

I. Those bacteria whose products have no effect upon temperature fluctuation.

II. Those whose products cause a slight and transient fever.

III. Those whose products cause a marked fever which lasts for many hours and possibly over night.

IV. Those whose products kill rabbits or produce collapse, etc.

The correlation between the physiological and a preliminary bacteriological grouping of the bacteria is contained in table 3.

It is interesting to note that all of the chromogenic bacteria fall within group I, or in other words do not form products in distilled water which produce fever when injected into rabbits. Only one strain—A12-1/9—of the non-chromogenic bacteria belonged to group I. The harmful organisms, i.e., those in groups III and IV, differ from those in groups I and II in their production of acid and coagulation in milk.

The two organisms whose products are lethal for rabbits, B36/4 and A31/1, are not outstanding in their cultural characteristics, except in one instance in the case of the former bacterium. At first milk was rendered acid three to four days after inoculation by B36/4 and there was definite peptonization, but after about 20 days an alkaline reaction was produced.

As mentioned previously, water 2a when first plated contained more organisms to 0.1 cc. than could be counted. Later the count decreased to 685 per cubic centimeter. Moreover, the three strains which represent all the different types of colonies in this water when isolated and tested produced no fever and fell within the chromogenic group. The fact that these were slow growers may account for the gradual loss of viable bacteria in the water. On the other hand the most harmful organisms were isolated from the most pyrogenic waters. These results therefore furnish ample evidence that bacteria are the source of pyrogen and moreover that only certain types of bacteria are responsible for the pyrogenic effect of waters.

Nature of pyrogen. As to the nature of the pyrogen, certain statements can be made. In the first place, it is destroyed by heat but very slowly; so slowly, in fact, that the destruction of the pyrogen in a highly potent water by autoclaving or boiling for fifteen minutes is scarcely noticeable as far as the physiological reaction is concerned. Therefore

for practical purposes the pyrogen is not destroyed by heat. Heating potent waters with either acid or alkali, however, does destroy pyrogen. The accompanying curves (fig. 1) illustrate these points. The mere addition of acid or alkali, however, does not inactivate a potent water, for 0.157 N-$NaHCO_3$ and 0.02 N-HCl solutions made with fever-producing water give marked rises in temperature.

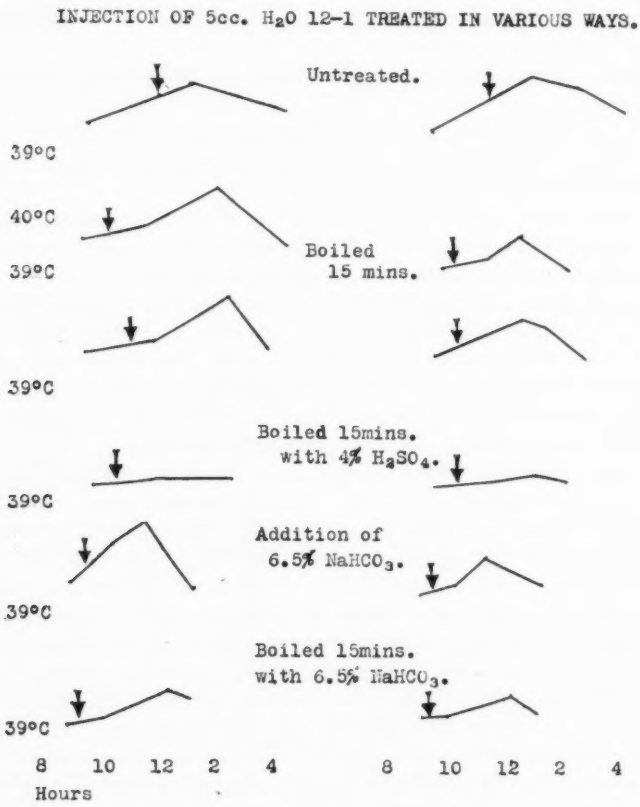


Fig. 1

Concentration of pyrogen. Concentration of the pyrogen is necessary for any chemical study since the amount present in distilled water is so very slight. It was found that with certain precautions it was possible to concentrate the pyrogen practically quantitatively. From table 4 it can be seen that in about 50 per cent of the trials the concentration by volume approximated the concentration by potency. In the other 50 per cent

of the cases considerable potency was lost during the concentration and this loss, it would seem, can be ascribed to one or more of the following causes. In the first place, since some pyrogen is destroyed by heat the best results were obtained when a small amount, i.e., no more than 1 liter, was distilled at a time, of course under diminished temperature and pressure. The time involved in the distillation had perhaps the most important bearing upon the results. For example, in any case where the water was exposed to the low temperature and pressure for more than four hours, most of the potency was lost. Furthermore, the loss in potency increased as the higher concentrations were reached, and in no case did it seem possible by this method to concentrate beyond the point where 0.01 cc. produced the minimal fever.

TABLE 4

Concentration of pyrogen

H_2O	CONCENTRATION BY VOLUME	CONCENTRATION BY POTENCY	ORIGINAL VOLUME	FINAL VOLUME	ORIGINAL MINIMUM FEVER DOSE	FINAL MINIMUM FEVER DOSE	TECHNIQUE OF CONCENTRATION			DATE
							Time	Temperature	Pressure	
			cc.	*cc.*	*cc.*	*cc.*	*hrs.*	*°C.*	*mm.*	
Tap 1b	3	3	1200	500	3	1	$2\frac{1}{2}$	60–70	20–40	10–25–23
1b1	10	10	500	50	1	$\frac{1}{10}$	3	50	10	11– 1–23
1b6	20	<3	2000	100	3		8–10	45	10–15	11–21–23
Dist. 10a	13	20	400	30	4		$4\frac{1}{2}$	40–50	10–15	12–15–23
12a	10	<2	2000	200	$\frac{1}{2}$	$>\frac{1}{10}$	10	45	15–20	11– 9–23
12a1	2–3	2	200	75	$>\frac{10}{75}$	$\frac{1}{10}$	4	45	10	11–15–23
12-1	7	5–6	500	70	4	$\frac{3}{4}$	$4\frac{1}{2}$	40–50	10–15	12–20–23
31	16.6	10	3750	225	$\frac{1}{2}$	$\frac{1}{30}$	4–5	50–60	12	1–10–24
31a	71.0	50	2490	35	$\frac{1}{2}$	$\frac{1}{150}$	$\frac{3}{4}$	50–60	12	1–18–24
			= (150 cc. 31)							
36a	53	4	4000	75	$\frac{1}{8}$	$\frac{1}{30}$	$4\frac{1}{4}$	50–65	15	2–14–24

No more striking evidence can be given for the quantitativeness and delicacy of the fever reaction in rabbits than the experimental data from which the above table was compiled. For this reason, three experiments cited in table 4 are given graphically in figures 2, 3 and 4.

It is clear that the height and length of the peak of the fever curve are more or less in proportion to the amount of material injected and with the smaller amounts the curve flattens out, making it fairly easy to determine the minimum amount of a substance which will just produce fever. After the maximum temperature rise is once reached, however, larger doses merely increase the duration of the rise rather than the height of the curve.

The reliability of the method is borne out by the following facts. In the case of water 31 and its concentrates 31A (concentrated 16.6 times) and 31Al (concentrated 71 times) it can be seen that animals 56, 61, 57

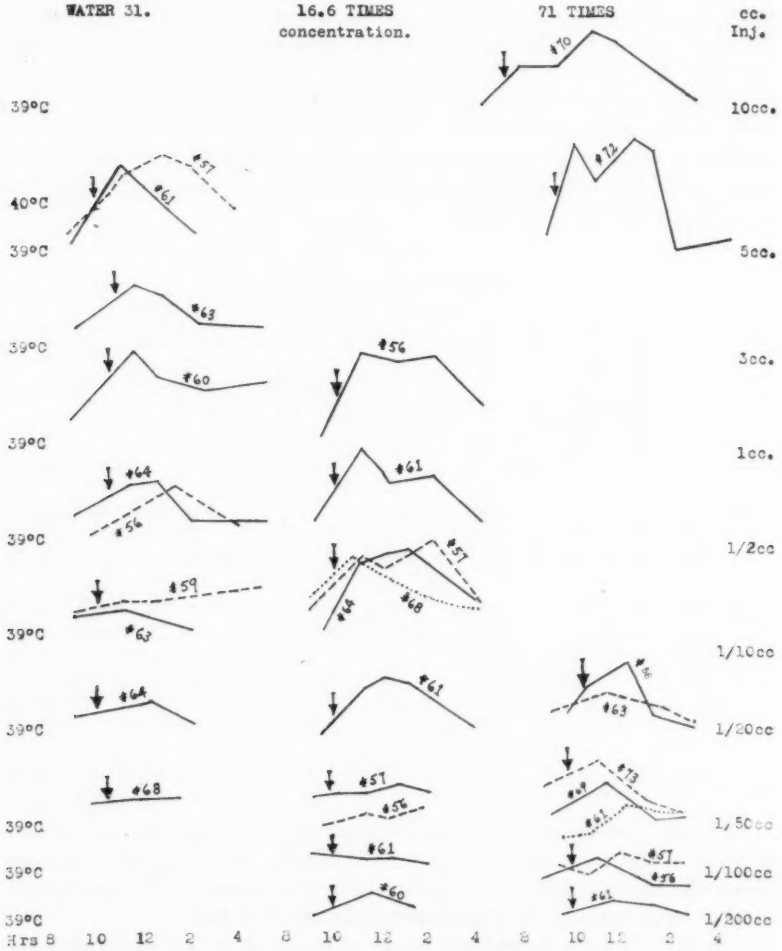


Fig. 2

reacted according to the dose given rather than because of individual idiosyncracies. For example, animal 56 gave marked reactions with $\frac{1}{2}$ cc. of water 31, with 1 cc. of water 31A and with $\frac{1}{20}$ cc. of water 31Al, but a very slight reaction with $\frac{1}{50}$ cc. 31A and $\frac{1}{100}$ cc. 31Al. Similarly animal

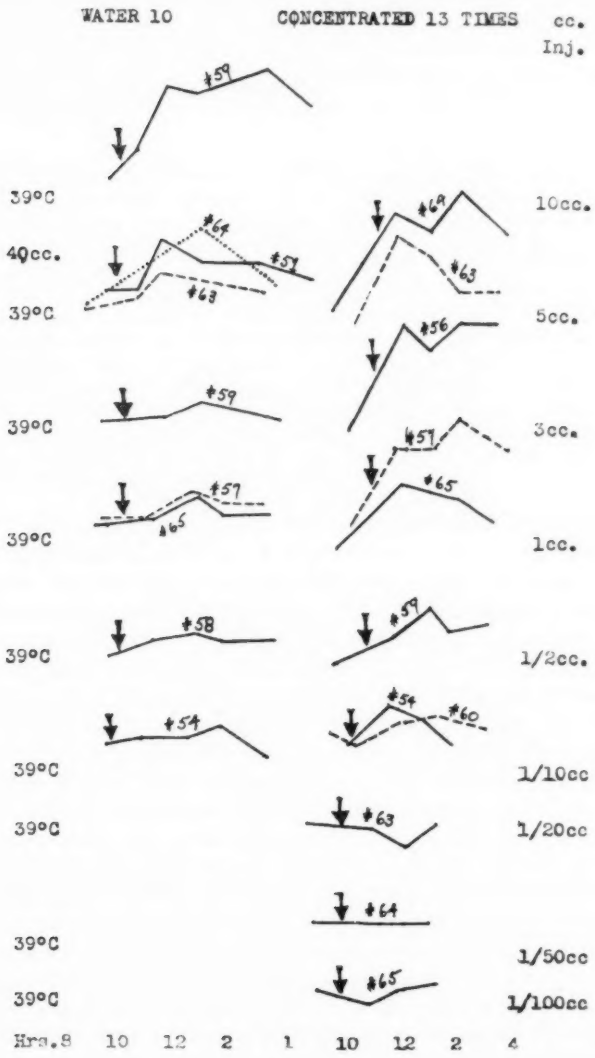

WATER 10
CONCENTRATED 13 TIMES
cc. Inj.
#59
39°C
#64
40cc.
#57
#63
39°C
#59
39°C
#57
#65
39°C
#58
39°C
#54
39°C
39°C
#69
#63
#56
#57
#65
#59
#54
#60
#63
#64
#65
10cc.
5cc.
3cc.
1cc.
1/2cc.
1/10cc
1/20cc
1/50cc
1/100cc
Hrs. 8 10 12 2 1 10 12 2 4

Fig. 3

57 gave marked reactions with 5 cc. of water 31 and $\frac{1}{10}$ cc. water 31A, but scarcely any reaction with $\frac{1}{50}$ cc. 31A and with $\frac{1}{100}$ cc. 31Al. Any number of similar cases can be studied.

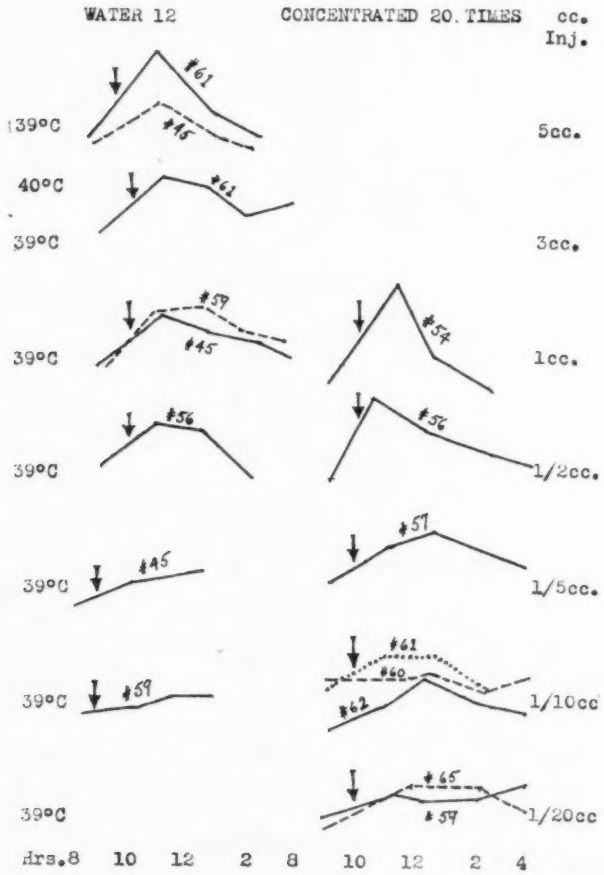


Fig. 4

Nitrogen content of waters and bacterial extracts. Since the pyrogen is a somewhat stable substance which can be concentrated, it might be possible to secure sufficient material for chemical study. The first natural question to arise is whether or not the pyrogen is protein or at least a nitrogenous compound.

A micro-nitrogen method[2] was devised whereby the amount of nitrogen in these water concentrates and in the Berkefeld filtrates of the bacterial extracts could be measured. This method combined the technics of the hydrogen peroxide digestion described by McMeekan and Koch (5), the distillation of the ammonia through a modified Folin (6) urea distillation tube, devised by Koch, and nesslerization in Nessler tubes (7). It is as follows:

Twenty-five cubic centimeters of filtered sterile extract or of water concentrate were boiled in a large pyrex test tube with 1 cc. of 1:1 H_2SO_4 until white fumes appeared and until the solution was charred. Then one to two drops of 5 per cent H_2O_2 (redistilled) were added to the warm digest and immediately a clear completely digested solution resulted. After cooling, 10 cc. of freshly redistilled water were added and then 5 cc. of saturated NaOH layered on. The liberated ammonia was then distilled into 25 cc. freshly distilled water containing 1 cc. 0.1 N H_2SO_4. After the distillate was cool it was nesslerized with 2.5 cc. Nessler solution and made to 50 cc. in a Nessler tube. It was found by several tests that all the ammonia was distilled over by the first distillation, and also that the addition of larger amounts of acid and of Nessler solution did not increase the ammonia yield. These solutions were then compared in a Nessler camera with standards containing varying amounts of an $(NH_4)_2\ SO_4$ solution of which 1 cc. was equivalent to 0.02 mgm. nitrogen, plus 1 cc. 0.1 N H_2SO_4 and 2.5 cc. Nessler solution.

Only freshly distilled water was used throughout and all the apparatus was carefully rinsed with it before use. Control tests on the freshly distilled water and the H_2O_2 were run with every series of determinations. Twenty-five cubic centimeter portions of the freshly distilled water were tested on nine different occasions and an average amount of 0.01 mgm. of nitrogen was detected in these blank tests. This accounts for the nitrogen in the H_2SO_4 and H_2O_2 used for digestion as well as for the amount in the 25 cc. of water. The total blank of 0.01 mgm. nitrogen was therefore subtracted from each result obtained on the concentrated waters and on the bacterial filtrates, so that the numbers reported in table 3 represent the actual amounts of nitrogen in the substances tested.

In order to settle the question as to whether or not the nitrogen in the bacterial extracts might be due to ammonia absorbed from the air because the suspension stood a day or more before filtration and also to settle the question as to whether the nitrogen in the Berkefeld filtrates was due to a substance washed from the candle, the following experiment was devised. An amount of freshly distilled water was incubated for the same length of time as the bacterial suspensions and then it was filtered through a Berkefeld candle. Twenty-five cubic centimeters of this filtrate contained the same amount of nitrogen as the previous blanks, proving therefore that these factors do not play a rôle.

[2] I wish to express my appreciation to Prof. Fred Koch for his suggestions and the use of his laboratory in carrying out the nitrogen determinations.

The numbers of milligrams nitrogen in 5 cc. of the various sterile bacterial extract, shown in table 3, indicate that there is no direct relationship between amount of nitrogen in a filtrate and the degree of fever produced by the intravenous injection of the material. On the other hand, the fever may be due to specific nitrogenous compounds in the filtrates in the same way that the fever produced by the water is due to products formed by specific organisms in the water.

Four liters of a fever-producing water were concentrated by the method previously described and found to contain 0.056 mgm. of nitrogen. Similarly 4 liters of a non-fever-producing water were concentrated and 0.020 mgm. of nitrogen found, leaving a difference of 0.036 mgm. nitrogen, which may or may not be responsible for the pyrogenic effect of the former water. One-tenth of a cubic centimeter of this pyrogenic water produced a minimum fever when injected intravenously into rabbits and, calculated as protein on the basis of the figures obtained, it would have a content of 0.000000005 gram protein per 0.1 cc.

The question as to whether or not the pyrogen is protein is a pertinent one. Ninhydrin tests on one liter concentrates of a highly potent water were negative, as were also the tests on several bacterial filtrates. But since the delicacy of this test is one part in 10,000 and since six parts protein per million, calculated from the nitrogen found, can be detected by the nitrogen method, we can not yet say that the pyrogen is not protein. Further micro-methods are needed in order to prove the nature of the nitrogenous compounds which have been shown to be present in distilled waters.

As can be seen from the figures quoted, the task of concentrating sufficient pyrogen for examination from water is almost an impossible one at the present time. Therefore, it seemed probable that at present more information concerning the nature of the pyrogen could be gained through serological tests than in any other way.

Immunological studies. Shock reactions with water. A few typical shock experiments were performed with a water concentrate, i.e., with 31Al which was water 31 concentrated 71 times.

A rabbit, no. 69, was injected with $\frac{1}{5}\frac{1}{0}$ cc. water 31A1 on January 19 and reacted with a slight rise in temperature of 0.7°C. On February 2 it was injected with 10 cc. 31A1. Within one hour and ten minutes it developed typical shock symptoms, i.e., scratched its nose, fell to one side paralyzed, collapsed, expelled bloody urine, gasped for air and died with violent convulsions. Autopsy revealed an enlarged right heart and congested liver.

On January 21, 5 cc. of this water injected into a new unused rabbit produced a fever of 2.2°C. but no reaction as described above.

On January 23, 10 cc. of the water produced a fever of 1.5°C. and slight collapse when injected into a rabbit that had received only one previous injection, and that, a freshly distilled water which produced no fever.

This experiment was repeated with a water concentrate 31X, of which $\frac{1}{5}\frac{1}{0}$ cc. produced a minimal fever.

When 10 cc. were injected intravenously into rabbit 77, which had received an injection of $\frac{1}{5}\frac{1}{0}$ cc. of the same water just two weeks previously, a typical shock reaction resulted. After one hour the animal became uneasy and moved about its cage continually and in thirty minutes more it was completely collapsed and the temperature gradually fell to 37.8°C., or a fall of 2.2°C. After two hours there was a slow recovery, the animal was able to sit up and the temperature rose to 41.2°C. which was 1.5°C. above normal. Finally the temperature decreased to normal.

Fifteen cubic centimeters of the same water injected into rabbit 75 which had also been injected two weeks previously with $\frac{1}{5}\frac{1}{0}$ cc., gave a rise in temperature of 1.4°C. in one hour and ten minutes. Bloody urine was passed and in five more minutes the animal was completely collapsed. The temperature gradually decreased until in three hours and ten minutes death occurred. Autopsy revealed an enlarged right heart and congested liver.

Fifteen cubic centimeters injected into rabbit 81 which had received no previous injection produced no visible reaction. Its temperature rose 0.7°C., then fell to normal in two hours, and rose again 1.1°C. above normal.

Water 41 was similarly concentrated until $\frac{1}{1}\frac{1}{0}\frac{1}{0}$ cc. gave a temperature rise.

Rabbit 102 when injected with 15 cc. of water concentrate 41A two weeks after receiving $\frac{1}{1}\frac{1}{0}\frac{1}{0}$ cc., gave a marked reaction but was never completely collapsed. A normal unused rabbit responded to 10 cc. with a fever but no visible reaction.

Another unused rabbit became slightly restless after a 15 cc. injection.

Rabbit 101 received a preliminary injection of $\frac{1}{1}\frac{1}{0}\frac{1}{0}$ cc. and three weeks later 10 cc. It responded to this last injection with a fever of 2.2°C. and a muddy fluid excretion.

The results with this water are not so striking, though suggestive of the definite reactions obtained in rabbits sensitized with the two former water concentrates.

These few experiments are suggestive of the production of anaphylaxis with bacterial proteins or at least with some product of bacterial nature.

A toxic bacterial product. Similar reactions and death were obtained with bacterial extracts—B36/4 and A31/1—and therefore much further work was done with the former material. Ten different lots of B36/4 extract were prepared at different times by suspending five slants in about 250 cc. freshly distilled water, incubating at 37°C. over night and then filtering through a tested Berkefeld.

The first two injections with the first preparation were followed by dramatic results. Within one hour from the injection both animals became uneasy, then limp; they fell to one side, defecated violently, breathed hard, were seized with convulsions and in fifteen minutes more were dead. As can be seen from the following table 5, deaths resulted in certain animals following the injection of preparations I, II, IV, V, VI and VII.

TABLE 5

LOT OF B36/4	DATE OF INJECTION	DEATHS	TIME	UNEASY, LONG FEVER, COLLAPSE, DIARRHEA	UNEASY, LONG FEVER, SLIGHT COLLAPSE?	UNEASY, LONG OR SHORT FEVER, NO REACTION	AMOUNT INJECTED	REMARKS
	1924	*Rabbit number.*					*cc.*	
	2-23	♯57	1 hour 15 minutes				5	1 slant incubated one day
		♯54	1 hour 15 minutes				5	
I	2-27				♯80 (new)—1.5°		5	
					♯68 —1.5°		5	
	2-28					♯60—0.5°	10	
II	2-29					♯60—0.8°	5	2 slants incubated one day
						♯80—1.5°	5	
III	3-6	♯70	Immediately	♯78—1.2°	♯58 —1.0°		10	5 slants incubated five days
	3-22	♯45	Next day				15	Sensitized on 6th with $\frac{1}{100}$ cc.
	3-12				♯81 —1.4°		5	5 slants incubated two days
	3-12				♯77—fall 0.6°		5	
	3-25			♯74—1.1°	♯77— 1.4°		5	
					♯58— 1.4°		15	
IV	3-26	♯84 (new)	1 hour	♯76—fall—1.3°			15	
	3-28			♯87—1.8°			5	
	4-5			♯88—1.1° (died 17th)			9	

V	4–5	#92 (new)	1 hour	#77—1.4°			7	5 slants incubated 4 days at 37°C. and 8 days at 20°C.
	4–10			#89—1.2°-3.2° fall (new)			1	
	4–18					#93—1.2° (new)	6	Autoclaved
	4–19				#90 (new)—1.0°	#74—1.3° autoclaved	8	
VI	5–8			#90—1.4°	#105 (new)—2.0°		5	5 slants incubated 12 days subcutaneously
					#106 (new)—1.0°		10	
					#107 (new)—0.25°		15	
		#76 (sick)	Immediately				7	
VII	5–10	#112	Next day	#108 (new)			10 12–15	5 slants incubated 5 days
VIII	5–12				#109 (new)		5	5 slants incubated 1 day
	5–20				#61—1.1°		10	
					#83—0.9°		10	
IX	6–3				#74—1.0°		10	5 slants incubated 3 days
					#116 (new)—1.45°		10	
A 31/1								
I	3–25			#78—1.0° (died in 13 days)			5	
	3–26	#56—1.5°	2 hours 30 minutes			#80—1.5°	5	
						#77—2.0°	5	
II	4–19	#91—1.0° (new)	1 hour 20 minutes		#76—1.7°		8	
		#93—0.3°	45 minutes	#95 (new)—1.6°			8	Autoclaved
	4–22				#82—1.9°		10	
						#96 (new)—1.8°	30	Subcutaneously

In the case of lots IV and V it seemed that new animals were killed by the injections whereas used animals were not. But this was not confirmed with lots VI, VII, VIII and IX. The majority of results indicate that the potency of the material deteriorates on standing and this fact might explain a few of the inconsistent results obtained. (There is evidence also that the pyrogen in water deteriorates on standing.) On the other hand, autoclaving extract V did not decrease its potency. The results with lot II of extract A31/1 were even more striking in this respect, since fresh material even after autoclaving produced death upon injection. The time of incubation of the bacterial suspensions before filtration was varied from one day to twelve days but this did not seem to be the determining factor for potency.

In general the later preparations of B36/4 extracts were the least potent and it was thought that possibly continued cultivation upon nutrient agar might have caused the organism to lose its power of producing toxic substances. Therefore, the original water 36 from which this organism was isolated was again plated and colonies selected which looked most like the B36/4 colony. Pure cultures were made from two of these colonies and their extracts were prepared in the usual way. A 10 cc. inoculation into a new animal caused death in one hour and fifteen minutes, and collapse in another used animal. Two more animals, one of which was a new one, inoculated later in the day responded with only moderate reactions. The similarity of these physiological reactions obtained by this organism to that obtained by B36/4, along with similarity in the bacteriological tests made, seems to indicate that the same organism was living in the water four months after the first isolation. But the physiological responses would not yet warrant a conclusion that cultivation in distilled water produced an organism with more toxic products than cultivation on agar.

Antibody production. A few immunological reactions of this organism were studied. Five animals 107, 97, 90, SG2 and SG3 were immunized by intravenous injections of suspensions of the living organism, starting with 1/50th slant and working up to 1/25th, 1/4th, 1/2 and 3/4ths slant. The injections were given at two to three day intervals at first and finally lenghtened to five to seven day intervals. The injection of one whole slant into an animal killed it. As can be seen from the table 6, the serum of rabbit 107 in a dilution of 1:2000 agglutinated the organism in vitro. The immune sera of 97, SG2 and SG3 agglutinated the organism in dilutions as high as 1:800,000. On the other hand, there was no agglutination whatever of the serum of rabbit SG3 by six other organisms—B12/8, A12-1/9, B23 B/4, B20/4 B. typhosus, and B. coli, thus indicating the specificity of this agglutinin.

In order to determine whether the body would produce antibodies to the soluble products of these bacteria, two rabbits, 99 and 115, were immunized

with 10 cc. injections of the sterile Berkefeld filtrate of the water extract of the organism B36/4. The immune sera of both rabbits agglutinated the organism in a dilution of 1:40,000. But the serum of rabbit 99 did not agglutinate any of the following six organisms—B12/8, A12-1/9, B23B4, B20/4, B. typhosus and B. coli, indicating the specificity of the agglutinin. One preliminary complement fixation test showed some fixation by the immune serum of rabbit 99.

In order to determine whether antibodies are found in the blood after the injection of contaminated distilled water, two rabbits' 102 and 103, were immunized with the concentrate of water 41. Four liters of this water were concentrated under the conditions described above to 225 cc., and 10–15 cc. of this concentrate, kept under sterile conditions, were injected intravenously at two to three day intervals. As seen in the chart, these immune sera definitely agglutinated organism B36/4 in dilutions of 1:8000 and 1:2000 respectively.

Several of the animals displayed the presence of agglutinins in 1:20 and even 1:40 dilutions of the normal serum, that is, serum obtained before any injections were given, indicating the presence of non-specific agglutinins or the fact that the animals had naturally acquired a small degree of immunity to this organism. The later postulate is very probable since water is a constituent of the diet.

Toxin nature of pyrogen. In order to determine whether or not the toxic substance was a true toxin two tests were performed in which there was an attempt to find a substance in the sera of rabbits 99 and 97, at the time their agglutinating power was the highest, which would neutralize the toxicity of the B36/4 extract. One rabbit injected with 10 cc. of 9B36/4 extract plus 2 cc. of 99 immune serum displayed no reaction other than a marked fever, while on the same day two other rabbits injected with 10 cc. of the extract minus the serum became very quiet, partially collapsed and excreted a reddish-brown fluid. Several days later the same extract, to 10 cc. of which were added 5 cc. immune serum from no. 97 produced no reaction other than a temperature rise. These results, to be sure, are too few and too preliminary upon which to base any conclusions but are merely suggestive for further work.

Route of injection. All the results so far mentioned have been obtained by intravenous injection. However, the same substances injected either intraperitoneally or subcutaneously are followed by reactions similar to those obtained by intravenous injection but to a less degree and in some cases considerably delayed. For example, water 12-1 which produced about 1.3°C. rise in temperature when given intravenously, was followed by a smaller rise of 0.9°C. when given intraperitoneally and by almost no rise when given subcutaneously in the same dose. Water 41 filtered and autoclaved gave practically the same rise in temperature when 15 cc. were

TABLE

Agglutination

SERUM DILUTION	RABBIT 99				RABBIT 115			RABBIT 190					RABBIT 103			
	5-20-24	5-31-24	6-5-24	6-13-24	5-27-24	6-5-24	6-13-24	4-30-24	5-3-24	5-29-24	6-5-24	6-13-24	5-10-24	5-20-24	6-5-24	6-13-24
1:20	−?	++			+	+	++	+	+		++	++	+	−	++	++
1:40	−?	++			−	+	++	−	−	−	++	++	+	−	++	++
1:80	−?	++	++		−	−	++	−	−	−	+	++	+?	−	+	++
1:200	−	++	++	++	−	−	++	−	−	−	+?	++	−	−	+?	++
1:400	−	++	+	++	−	−	++	−	−	−	+?	++	−	−	−?	++
1:800	−	++	−	++	−	−	++	−	−	−	+?	+	−	−	−	+
1:2000	−	++	−	++	−	−	++	−	−	−	+?	+	−	−	−	+
1:4000	−	++	−	++	−	−	++	−	−	−	−	+	−	−	−	+?
1:8000	−	++	−	+	−	−	+	−	−	−	−	+	−	−	−	+?
1:20,000			−	+		−	+					−				−
1:40,000			−	+		−	+					−				−
1:80,000			−	−		−	+					−				−
1:200,000			−	−		−	−					−				−
1:400,000			−	−		−	−					−				−
1:800,000			−	−		−	−					−				−
Injections intravenous	10 cc. B36/4 filtrate				10 cc. B36/4 filtrate			10-15 cc. H_2O-41A					10-15 cc. H_2O-41A			
	1924				*1924*			*1924*					*1924*			
	5-20				5-29			5-12					4-30			
	23				31			29					5-13			
	26							31					26			
	29												29			
	31												31			
	6- 2				6- 2			6- 2					6- 2			
	5				5			5					5			
	7				7			7					7			
	10				10			10					10			

given subcutaneously or intraperitoneally as when 5 cc. were given intravenously.

Thirty cubic centimeters of extract 2A31/1 produced 1.8°C. rise when given subcutaneously. Similarly 10 cc. of extract 6B36/4 given subcutaneously was followed by a rise of 1.0°C. A whole slant of B36/4 when injected either subcutaneously or intraperitoneally did not kill but pro-

6
of B36/4

RABBIT 107			RABBIT 97						RABBIT 90					RABBIT SG2						RABBIT SG3					
5-12-24	5-31-24	6-1-24	5-8-24	5-12-24	5-28-24	5-31-24	6-5-24	6-13-24	5-10-24	5-20-24	5-20-24	6-5-24	6-13-24	5-14-24	5-21-24	5-26-24	6-2-24	6-4-24	6-13-24	5-14-24	5-21-24	5-28-24	6-2-24	6-4-24	6-13-24
–	++	++	+	+	+?	++			+?	+?	++		+	–	–	+	+	+	++	–	+	–	+	+?	++
–	++	++	–	+	+	++			–	+?	+			–	–	+	+	+	++	–	–	–	+	+?	++
–	++	++	–	–	+	++			–	+?	+			–	–	–	+	+	++	–	–	–	+	+?	++
–	++	++	–	–	+	++	++	++	–	+?	–	+?	++	–	–	–	+	+	++	–	–	–	+	+?	++
–	++	++	–	–	–?	++	++	++	–	+?	–	–	+	–	–	–	+	+	++	–	–	–	+	–	++
–	++	+	–	–	–	++	++	+	–	–	–	–	+	–	–	–	+	+	++	–	–	–	+	–	++
–	++	+	–	–	–	++	++	++	–	–	–	–	+				+?	–	++	–	–	–	+	–	+
–	++	–	–	–	–	++	+?	++	–	–	–	–	+				–	–	++	–	–	–	+	–	+
–	++	–	–	–	–	++	+?	++	–	–	–	–	+				–	–	++	–	–	–	–	–	+
							+?	+				–	–				–	–	+				–	–	+?
							+?	+				–	–				–	–	+				–	–	+
							–	+				–	–				–	–	+				–	–	+
							–	+				–	–						+						+
							–	+				–	–						+						+?
							–	+				–	–						+						–

B36/4 suspension		B36/4 Suspension		B36/4 suspension		B36/4 suspension		B36/4 suspension	
Date	Slant	Date	Amount	Date	Amount	Date	Slant	Date	Slant
1924									
5-10	1/50	5- 8	1/50	5-10	1/25	5-14	1/4	5-14	1/4
-12	1/25	5-10	1/25	5-12	1/25	5-21	1/4	5-21	1/4
-26	1/5	5-12	1/25	6- 2	1/4	5-23	1/2	5-23	1/2
-28	1/4	5-26	1/5	6- 5	1/2	5-26	1/2	5-26	1/2
-30	1/5	5-28	1/4	6- 7	3/4	6- 2	1/2	6- 2	1/2
6- 1	1/4	5-30	1/5	6-10	3/4	6- 4	1/2	6- 4	1/2
		6- 2	1/4						
		6- 5	1/2						
		6- 7	3/4						
		6-10	3/4						

duced a fever, and also a typical slight reaction, i.e., the animals were quiet and slightly collapsed.

Reactions in guinea pigs. All of the experiments so far reported have been done upon rabbits. The following figure 5 shows the daily normal temperature variations of 17 guinea pigs. The average variation of 0.62°C. is slightly higher than the average obtained in the rabbits studied, and

altogether the guinea pig did not seem to be as desirable for temperature studies as the rabbit. Figure 5 shows that similar temperature reactions can be produced in guinea pigs as in rabbits by injecting the same materials.

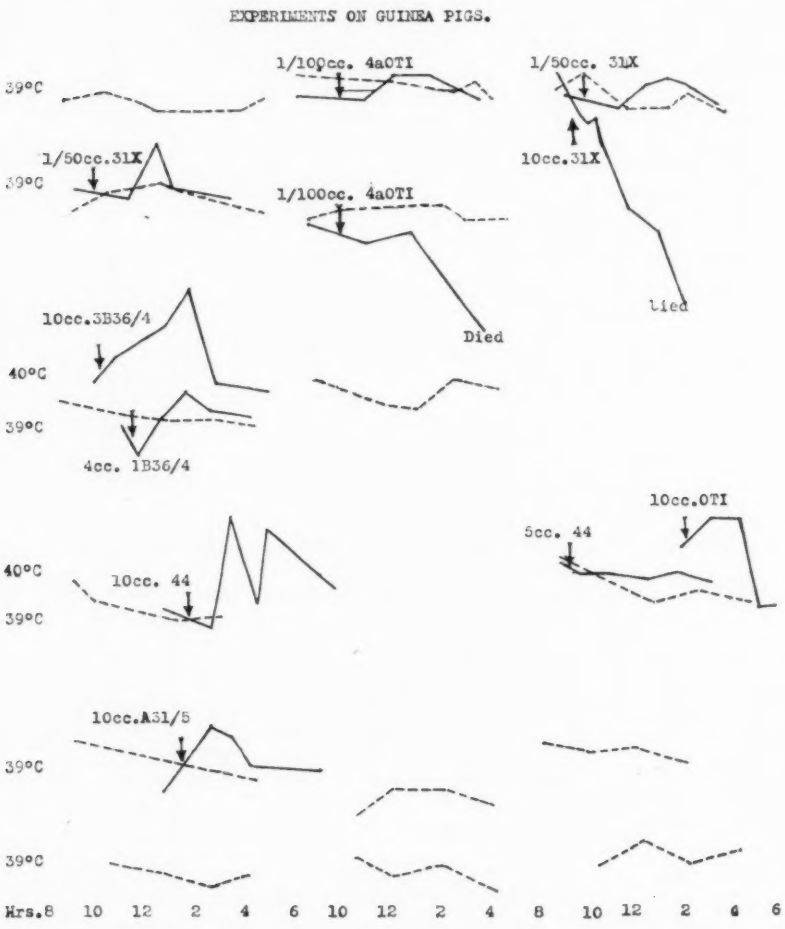


Fig. 5

The dotted lines represent the normal temperatures, and the complete lines represent reactions produced in the same animal by the intraperitoneal injection of a substance.

Reactions in mice. The temperature variations of mice can not be

readily determined but it is possible to produce evident reaction symptoms by intraperitoneal injections. For example, one experiment was performed in which two mice were inoculated with 1 cc. each of a freshly distilled water. They displayed no symptoms. Two more were inoculated with 1 cc. each of water 41, a pyrogenic water, and both became very quiet and their hair roughed. Two more were inoculated with 2 cc. each of 4B36/4 and these mice at first were uneasy and then quiet but livened up within the hour. Later two mice were inoculated with 2 cc. of extract D36/3 and one with 1 cc. All three were quiet and displayed roughed hair and jerky motions. One died within two days and a second within five days.

Reactions in man. More interesting and more important than the reactions obtained in animals are the responses of man to the same material. A few preliminary tests of this nature were made. Patients in the Cook County Hospital were selected who had given a history of normal temperatures.[3]

The first series of experiments consisted in 5 cc. subcutaneous injections of distilled water into the arms of four men. Two men, a normal and a diabetic, received a freshly distilled and autoclaved water which produced no fever in rabbits. Two others, a patient with gastric ulcer and a patient who had been operated upon for appendicitis, received water Al, which had produced 0.8°C. and 1.5°C. rise in temperature in rabbits the day before. Both waters when plated for presence of bacteria were sterile. No rise in temperature was produced in any of these four men.

The second series of experiments consisted in three intravenous injections of 5 cc. amounts, but this time the material was injected as normal saline. It had been shown in previous experiments upon rabbits that addition of salt to pyrogenic water neither increased nor diminished the effect of the pyrogen and therefore, in order to obviate confusion with effects due to hemolysis of the blood, all the waters injected intravenously were prepared as normal saline. Into patient 1, diagnosed as psychasthenic, 5 cc. of a saline made from a non-fever-producing water were injected into the basilary vein. Patient 11, suspected to have a stone in the right kidney, received saline made from water 41 (see table 11). Patient 111, diagnosed as post-operative ulcer, received 5 cc. of filtered saline extract of organism OTI, which produced fever in rabbits. All three solutions had been autoclaved and when plated were sterile. No temperature rise was obtained in any of these cases.

A third series of experiments consisted of three intravenous injections of 50 cc. amounts of saline made from freshly distilled water or water 41.

[3] I wish to express my appreciation to Dr. Karl Koessler through whom this work was made possible.

Patient 1, diagnosed as having an aneurism of the aorta, received autoclaved saline made from non-fever-producing water. Patient 11 (trigeminal neuralgia) received saline made from water 41 and sterilized by filtering through the Berkefeld candle. Patient 111, suspected of having a stone in the right kidney received saline made from water 41 and autoclaved. No temperature rises were obtained in these three cases.

A fourth series of experiments consisted of four intravenous injections by the gravity method of 85 cc. amounts of saline made from freshly distilled water and waters 23 and 36. Patient 1, diagnosed as having heart decomposition, received the saline made from freshly distilled water. Patient 11 (pyleonephritis and suspected of having cancer of the stomach) received saline made from water 36. Patient 111, (diffuse nephritis) received saline made from water 23 sterilized by autoclaving. Patient IV, tuberculosis suspect, received saline made from water 23, sterilized by filtering through the Berkefeld. The results in this series although unmistakably positive in some cases, can not be presented without some question because of the use of a defective thermometer. The control, patient 1, receiving saline made from non-fever-producing water must be practically discarded on this account. Patient 11, receiving saline 36 undoubtedly responded with a temperature rise of at least 0.9°C. and also had a chill, lasting twenty minutes (see fig. 6-a). Patient 111, receiving saline 23 autoclaved, most probably displayed some rise in temperature. There is no reliable evidence to indicate that patient IV, receiving saline 23 filtered, gave any fever.

A fifth series consisted in four intravenous injections of 80 to 100 cc. of saline made from non-fever-producing water and from waters 31, 33 and 41. These injections were given with a 100 cc. all glass syringe, thus eliminating chances for contamination possible by the gravity method. The autoclaved solutions were warmed to body temperature and injected very slowly. Patient 1 (aortic and mitral insufficiency) received 100 cc. of saline made from non-fever-producing water. The temperature rose 1.2°C. in four hours, but the patient had no unusual sensations (fig. 6-b). History of the case showed many previous temperatures between 37.2°C. and 37.8°C. but for four days prior to the injection the temperature had averaged 36.7°C. This same saline (5 cc.) produced $\begin{cases} 0.00° \\ 0.35° \end{cases}$C. rise in temperature in rabbits. Patient 11 (decompensation) received 90 cc. saline made from water 31 (fig. 6-c). In three and a half hours the temperature had risen 1.5°C. One hour after the injection the patient complained of being very cold, his lips were purple and there was some cyanosis. Five cubic centimeters produced 0.45°C. rise in temperature in a rabbit. Patient 111 (cancer of the esophagus, chronic myocarditis and arthritis) received 80 cc. of saline made from water 41

(fig. 6-d). Immediately the patient complained of being cold; his temperature was as low as 35.1°C. One hour and a half after the injection, he had a violent chill lasting ten minutes, and then the temperature rose to 1.0°C. above the average before the injection. A rise of 1.3°C. in temperature was produced by 5 cc. in a rabbit. Patient IV (ascites and cirrhosis of the liver) received 90 cc. saline made from water 33 (fig. 6-e).

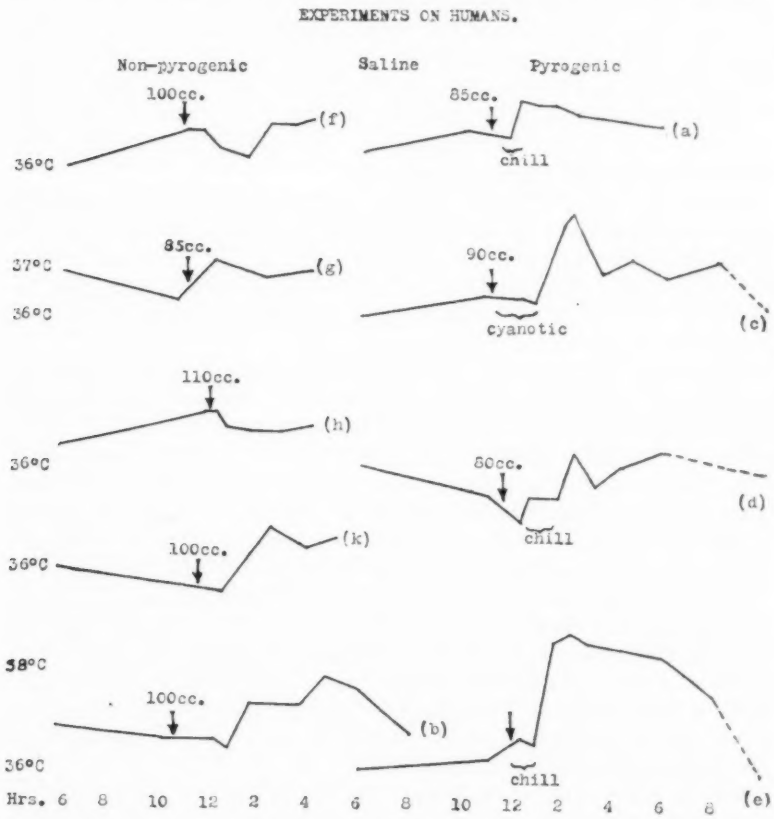


Fig. 6

One-half hour after the injection the patient had a violent chill which lasted one-half hour and then the temperature gradually arose to 38.8°C. or about 2.5°C. above the average pre-injection level. This saline (5 cc.) produced 1.3°C. rise in temperature in a rabbit.

A sixth series consisted in four intravenous injections of 85 to 110 cc. of saline made from non-fever-producing water, which caused no rise in

temperature in two rabbits in 5 cc. doses. Patient I (fig. 6-f) with peptic ulcer, received 100 cc.; patient II (g) with fixation of wrist joints received 85 cc.; patient III (h) with sciatica received 110 cc.; and patient IV (k) with cardiac decompensation and acute nephritis received 100 cc. In no case was there a reaction noticable either to an observer or to the patient himself. Moreover, there was not an abrupt rise in temperature as in the patients receiving saline made from pyrogenic waters.

These results therefore indicate that marked reactions, fever and chill, such as described in the literature following protein, sugar, salvarsan, etc., injections, can be obtained in man by the injection of sufficient amount of saline made with contaminated water. Moreover, such reactions can be avoided by using water purified by the method here described, in order to free it from pyrogenic material.

Dr. W. W. Duke, Kansas City, Mo., has informed me that since using this method of purifying water (by distillation) he has been able to eliminate reactions following intravenous injections of salvarsan or neosalvarsan even in his highly allergic patients. He also emphasizes the necessity of a trap in the distillation apparatus and is now using a Glinsky tube between the flask and condenser.

Discussion of results. The conclusions reached in this paper are based upon over 2000 experiments performed on 110 rabbits, in addition to other animals. It has long been known that certain distilled waters when injected intravenously into the body, produce a rise in temperature. The question as to the cause of these temperature fluctuations has been much debated. Many theories have been proposed, the most outstanding of these being that of inorganic or organic contamination of the water and that of change of physical state of the blood produced by the injections.

It may be true that hemolysis of the blood following the injection of large amounts of distilled water is deleterious. But it is also true that hemolysis is not the cause of the reactions mentioned in this paper, since identical results are obtained whether the solutions are or are not made isotonic with the blood. It is further true that the effect here described is not due to inorganic contamination of the water, nor to the hydrogen ion concentration of the solutions.

On the other hand, there seems to be no doubt, after studying these results, that the pyrogen is a bacterial contaminant, as suspected by many former investigators,—Hort and Penfold (8), Wechselmann (9), etc. It is possible to free distilled water of this contamination by a proper distillation and only such fresh distillates should ever be used in the preparation of solutions for intravenous injections. If these fresh distillates are sterilized immediately and preserved sterile they will remain free from pyrogen. On the other hand, if they are once allowed to become contaminated, it is not possible to free them of pyrogen by autoclaving, since

the pyrogen is very resistant to heat, although it is slowly destroyed. A bacterial count of a water may not be a true index of its pyrogenicity for this reason, as well as for the reason that only specific bacteria are responsible for the production of pyrogen.

By correlating a bacteriological study of the flora of twelve distilled waters and a study of the physiological reactions produced in rabbits by the injection of water extracts of these organisms, it is clear that the bacteria responsible for the production of pyrogen fall within a limited area of one group. They are all non-chromogenic bacteria and have the one outstanding common characteristic of acid production in milk. The significance of these facts needs further study. A detailed description of these organisms is given in Part II of this paper.

A convergence of such bacteriological and physiological studies and a study of the chemical nature of the compounds formed will be necessary for future progress in this field. Only the most elementary steps in the chemical investigation of the pyrogen have so far been taken. For example, the knowledge of its resistance to heat led to the possibility of concentrating the pyrogen to a certain extent under diminished temperature and pressure.

It has been shown that distilled water which has once been contaminated by these bacteria, even after filtering through the Berkefeld, contains considerable nitrogen. It still remains to prove by more delicate methods than now exist whether this nitrogen is in the form of protein or other nitrogenous compounds.

Typical shock reactions can be produced by these pyrogenic waters and by extracts of certain strains of the isolated bacteria. For example, animals which had been sensitized by the injection of a minute amount of the pyrogen, responded two weeks later to an injection of a large amount with typical anaphylactic-like reactions and even death, while animals not so sensitized did not do so. Similar reactions and death followed the first injection of Berkefeld filtrates of two strains of bacteria isolated.

Furthermore, specific serum antibodies of these bacterial products were produced. Agglutinins for one specific strain of bacteria were demonstrated in dilutions as high as 1:20,000 of serum from rabbits immunized with this bacterium, and also in rabbits immunized with sterile Berkefeld filtrates of aqueous suspensions of the organism. These agglutinins were shown to be specific for the strain of bacteria used for inoculation. Agglutinins were also demonstrated in serum dilutions of 1:2000 in rabbits immunized with distilled water shown to be contaminated with this organism.

As a result of these findings the necessity of considering and eliminating this very common contaminant of distilled waters must be emphasized in any experimental work involving intravenous injections. Especially

is this true in the immunological and serological researches where repeated injections are given and are followed by antibody production or shock reactions. Perhaps these facts may explain a few of the inconsistent results obtained by some investigators.

The delicacy and constancy of the temperature fluctuations presented in this paper are as gratifying as any biological tests can be expected to be and the rabbit is therefore a suitable animal for such studies. Guinea pigs were shown to give similar temperature reactions, and it was possible to observe a gross reaction in mice.

It was important to find that man reacts in the same way to the same material given in comparable doses. That is, an injection of 80 to 100 cc. of sterile saline made from distilled water in which bacteria had once been demonstrated, produced a violent chill, followed by a marked rise in temperature, as high as 38.8°C. in one case. On the other hand, injection of the same amount of saline made from non-fever-producing water, produced no reaction, indicating therefore that the method described is adequate for preparing a non-fever-producing saline.

These experiments, together with the fact that it was possible (2) to prepare a protein, namely casein, which produced no fever in rabbits, suggest an attack upon the problem of protein fevers. They also may serve as a suggestion for a means of eliminating many of the reactions following intravenous injections of large amounts of any solution, such as saline, salvarsan, drugs, sugar, etc.

SUMMARY

About 50 per cent of the distilled waters examined from different laboratories produce fever when injected intravenously into rabbits. The fever-producing material is a product formed by specific bacteria which contaminate the water, and can be removed from the water by distillation. It cannot be estimated by a bacterial count of the pyrogenic water.

Correlation between a bacteriological study of the flora of twelve distilled waters and a study of the physiological reactions produced in rabbits by the injection of aqueous extracts of the different strains of bacteria, revealed the fact that the bacteria responsible for the production of pyrogen fall within a limited area of the group of non-chromogenic bacteria described. They are, furthermore, characterized by the production of acid in milk. These bacteria are further subdivided into four groups according to the reactions produced by their extracts.

As to the chemical nature of the pyrogen, it is destroyed very slowly by heat alone, but readily by heat in the presence of acid or alkali. It can be concentrated under diminished temperature and pressure, the amount of concentration being tested by the degree of rise in temperature in rabbits.

Nitrogen has been demonstrated in these pyrogenic waters after con-

centration and also in aqueous bacterial extracts. However, no correlation could be demonstrated between the amount of nitrogen and the degree of pyrogenicity of a water or of an extract.

Anaphylactic reactions were produced in rabbits sensitized with concentrated pyrogenic waters, while non-sensitized rabbits did not respond similarly.

Similar shock reactions were produced in rabbits by the first injection of aqueous Berkefeld filtrates of two strains of bacteria isolated from pyrogenic waters. The toxicity of these substances decreased quickly on standing, but was not materially affected by autoclaving.

Agglutinins were produced to organism B36/4 in rabbits immunized with any of the following—suspensions of this organism, aqueous Berkefeld filtrates of the suspension, and concentrated pyrogenic waters. This agglutinin, however, was shown to be specific for organism B36/4.

Similar febrile reactions were obtained by subcutaneous and intraperitoneal injections but they were less marked than by intravenous injection.

Similar reactions were produced in guinea pigs by injection of pyrogenic waters and noticeable reactions occurred in mice.

Chill and fever were produced in man by the intravenous injection of 80 to 100 cc. of saline made from pyrogenic water, whereas no reaction followed the injection of saline made from freshly distilled water.

I wish to express my sincere appreciation to Prof. H. Gideon Wells for his helpful criticisms and inspiration throughout this investigation.

BIBLIOGRAPHY

(1) Seibert: This Journal, 1923, lvii, 90.
(2) Seibert and Mendel: This Journal, 1923, lvii, 105.
(3) Seibert and Mendel: This Journal, 1923, lvii, 83.
(4) Prescott and Winslow: Elements of water bacteriology, 1915.
(5) McMeekan and Koch: Journ. Amer. Chem. Soc., 1924, xlvi.
(6) Folin and Wu: Journ. Biol. Chem., 1919, xxxviii, 96.
(7) Tanner: Bacteriology and mycology of foods. 1919.
(8) Hort and Penfold: Brit. Med. Soc., 1911, xvi, 1589.
(9) Wechselmann: Münch. med. Wochenschr., 1911, viii, 1510.

THE CAUSE OF MANY FEBRILE REACTIONS FOLLOWING INTRAVENOUS INJECTIONS

II. The Bacteriology of Twelve Distilled Waters[1]

JANET M. BOURN and FLORENCE B. SEIBERT

From the Department of Pathology of the University of Chicago

Received for publication October 2, 1924

A brief survey of the bacteriology of the distilled waters whose pyrogenic properties were studied and reported in Part I of this paper is essential to locating the specific factors which are responsible for the production of the pyrogen. It has been pointed out that the mere presence of large numbers of bacteria in a water is not sufficient to render it fever-producing when injected into rabbits. On the other hand, it has also been shown that a water must at some time or other be contaminated with bacteria before it will become pyrogenic. Therefore, the pyrogenicity of a water would seem to depend upon the presence of specific strains of bacteria. The questions then arise as to whether only one strain of bacteria is responsible for the production of the pyrogen or whether a number of strains can give rise to this property, and if the latter, how closely these strains may be related. In order to answer these questions, a bacteriological study, which is here presented, was undertaken. It seems desirable to state in detail the numerous characteristics of the different bacteria isolated and studied, since these organisms are not commonly described in the literature.

Method of isolation. Twelve distilled waters were plated upon plain nutrient agar in suitable dilutions, the numbers of bacteria previously determined for each, and allowed to incubate at room temperature for several days. After typical colonies appeared, which in some cases required from four to seventeen days, an attempt was made to select from each plate or group of plates all representative strains of bacteria as determined solely by different colony characteristics. Pure cultures of these organisms were isolated on plain nutrient agar slants and a preliminary study of their cultural features and biochemical reactions was made as indicated in the accompanying table.

[1] The bacteriological studies were made possible through a grant from the Logan Fellowship given by the University of Chicago—1924. The paper itself is a continuation of the work done by Doctor Seibert as holder of the Porter Fellowship in Physiology.

Characteristics common to all of the bacteria. Certain characteristics stand out as being common to all the strains of bacteria studied in this connection. They were all able to live and reproduce in distilled water, and some were found to be viable in the original water many months after their initial isolation. They, therefore, appear to be organisms similar to the Prototrophic bacteria described by Prescott and Winslow (1), which are able to exist upon very simple constituents, possibly the gases of the air dissolved in the water, or upon the small amount of organic material afforded by their autolyzed predecessors.

They were all somewhat slow growers, requiring several days at least to reach a comparatively abundant growth at room temperature. The chromogenic bacteria were the slowest in this respect, appearing first as white colonies, which later developed their characteristic color, and it was not possible in some cases to cultivate these strains over long periods on plain sugar. All of the bacteria studied apparently grew well at room temperature, since it was from water standing at room temperature that they were isolated. Although this seemed to be the optimum temperature for them, they were able to grow for a while and more rapidly at 37°C. For example, two waters containing many living bacteria were shown to be sterile after incubation at 37°C. for one month, while the same waters maintained at 20°C. over the same period exhibited the presence of viable organisms.

No strain liquefied gelatin nor produced indole. Fermentation of dextrose, lactose or saccharose broth with the production of acid and gas was not observed. In one instance, however, acid but no gas was produced in dextrose broth after seven days' incubation at 37°C. The majority of the organisms, with but two exceptions, were small Gram-negative bacilli. Other characteristics, such as motility, reduction of nitrates, growth in plain nutrient broth and on raw potato, reaction in milk, type of growth in gelatin and on agar were variable. As already indicated, the one property which seemed to characterize those strains producing considerable pyrogen was the production of acid and coagulation in milk.

Some facts in regard to organism B36/4. This organism, as seen from Part I, was characterized especially by the production of an exceptionally toxic pyrogen and for this reason was studied a little more in detail than some of the other bacteria. However, the only outstanding cultural characteristic which serves to differentiate it from the other pyrogen-producers is the reaction in milk. Three to four days after inoculation the milk was rendered acid and there was definite peptonization, with a secondary alkalinity taking place after about twenty days.

Aversion to the presence of organic matter is clearly demonstrated by experiments upon this organism. It was shown to multiply rapidly in doubly distilled water four months after it was first isolated. Sterile

STRAINS ISOLATED	AGAR SLANT	POTATO DEXTROSE AGAR SLANT	POTATO SLANT	GELATIN STAB			DEXTROSE AGAR STAB				NUTRIENT BROTH				BROM CRESOL MILK					FERMENTATION BROTH ACID AND GAS		
				Liquefaction	Stab growth	Surface growth	Gas production	Stab growth	Colorless surface growth	Pink surface growth	Turbid	Pellicle	Flocculent growth	Mucoid threads	Acidity	Coagulation	Alkalinity	Peptonization	Secondary alkalinity	Dextrose	Lactose	Saccarose
OCI	Bright pink, confluent, moist	Bright pink, glistening, moderate	Rose red	–	+	–	No apparent growth				+						+–?			–	–	–
D2a/2	Confluent, semi-translucent, moist	Slightly pink, semi-translucent, moderate	Tan; change to rose brown	–	+	–	–	+	+	*	+						++			–	–	–
D2a/1	Confluent, semi-translucent, moist	Slightly pink, semi-translucent, moderate	Tan, glistening, raised	–	+	–	–	+	+	*	+						++			–	–	–
D2a/3	Confluent, semi-translucent, moist	Slightly pink, semi-translucent, moderate	Pale pink	–	+	–	–	+	+	*	+						++			–	–	–
B10/7	Semi-translucent, moist, confluent	Slightly pink, semi-translucent, moderate	Tan brown	–	+	–	–	+	+	*	+						++			–	–	–
B36/5	Deep yellow, confluent, moist	Pale yellow, slimy, semi-translucent	No apparent growth	–	+	–	No apparent growth				? Growth †				No change					–	–	–
B12/3	Deep yellow, confluent, moist																					
A31/4	Deep yellow, confluent, moist																					
A31/6	Deep yellow, confluent, moist																					
OCIII	White, dry, dull; moist, massed growth	Flesh colored, dry; later moist growth	Flesh colored, raised, papillary surface	–	+	+‡	–	+				+					++			–	–	–
B8/6	Slightly pink, semi-translucent, confluent	No growth	No apparent growth	–	+	+–**	? Growth				? Growth††				No change					–	–	–
B12-2/6	Heavy, gelatinous semi-translucent	Heavy, opaque, color pink	Dark, reddish brown, raised	–	+	–	–	+		+	+	+					+	+		–	–	–

A12-1/8	Heavy, gelatinous semi-translucent	Heavy, flesh-colored, opaque	Rose pink; change to ruddy brown, heavy, raised	−	+	+	−	+		+	+	+					+	+		−	−	−
B12-2/4	Semi-translucent discrete colonies	Heavy, colorless	No apparent growth	−	+	−	−	+	+		+		+	+			+−?			−	−	−
A12-1/9	Light, translucent, confluent	Heavy, opaque, colorless	Delicate brownish gray	−	+	−	−	+	+		+		+				+−			−	−	−
B36R/4	Translucent, discrete, minute colonies	Yellow colonies and confluent colorless growth	No apparent growth	−	+	−	−	+				No apparent growth‡‡					No change			−	−	−
B12/8	Heavy, semi-translucent	Heavy, flesh-colored opaque	Pale pink; change to ruddy brown	−	+	−	−	+		+	+	+					+	+		−	−	−
B36/4	Heavy, semi-translucent	Heavy, colorless	No apparent growth	−	+	−	−	+	+			+	+		+			+	+	−	−	−
B10/8	Heavy, gelatinous, semi-translucent	Heavy, cream-colored, opaque	Light brown, heavy, moist	−	+	−	−	+		+	+	+		+	+	+		+		−	−	−
B23B/4	Heavy, gelatinous, semi-translucent	Heavy, flesh-colored, opaque	Yellowish brown	−	+	−	−	+		+	+				+	+		+		−	−	−
B20/4	Heavy, gelatinous, semi-translucent	Heavy, flesh-colored, opaque	Yellowish brown	−	+	−	−	+		+	+				+	+		+		−	−	−
B23B/1	Heavy, gelatinous, semi-translucent	Heavy, flesh-colored, opaque	Yellowish brown	−	+	+−	−	+		+	+	+		+	+	+		+		−	−	−
B20/1	Heavy, gelatinous, semi-translucent	Heavy, flesh-colored, opaque	Vivid brown, glistening, raised	−	+	−	−	+		+	+	+		+	+	+		+		−	−	−
B23A/1	Heavy, fluorescent	Heavy, flesh-colored, opaque	Brown, raised, heavy	−	+	−	−	+		+	+			+	+	+				−	−	−
A31/1	Heavy, gelatinous, semi-translucent	Heavy, opaque, colorless	Yellowish, tan, moist	−	+	−	−	+		+	+	+		+	+	+				−	−	−
A31/5	Heavy, gelatinous, semi-translucent	Heavy, flesh-colored, opaque	Dull pink; change to cocoa brown; heavy raised	−	+	−	−	+		+	+			+	+	+				+§§	−	−
OTI	Heavy, semi-translucent	Heavy, flesh-colored, opaque	Dark brown, heavy, raised, glistening	−	+	−	−	+		+	+	+		+	+	+		+		−	−	−

* Characteristic pink "water falls" growth just below surface of stab extending less than ½ inch down, and intense pink ring at edge of surface growth.
† Growth evident in peptone solution and nitrate solution; former slightly turbid with yellow ring at surface.
‡ Nail head surface growth with pale pink center.
§ Subsequent transfers exhibited bright pink surface growths.
** +− signifies slight growth; wedge-shaped rose pink stab growth.
‡‡ Slight turbidity in peptone and nitrate solutions.
§§ Acid, no gas, produced after 7 days' incubation.

CLASSIFICATION ACCORDING TO JORDAN	SOMEWHAT SIMILAR TO ORGANISM	GROUP PROPERTIES	STRAINS ISOLATED	FEVER GROUP	NUMBER OF H_2O SOURCE	MORPHOLOGY	MOTILITY	AGAR COLONIES	INDOLE	NITRATES REDUCED
1. Group XIII:										
A. Red chromogenic bacilli Type—B. ruber	Bac. roseus	Non-motile Pink pig. Milk alk. Gel. not liq. Nitrates not red.	OCI* (Gram +)	I		Gram positive bacilli: endospores	–		–	–
			D2a/2	I	2a	Gram negative bacilli; pleomorphic	–	Round, moist, pink-centered; surface	–	–
	B. ruber	Motile Pink pig. Milk alk. Gel. not liq. Nitrates not red.	D2a/1	I	2a	Gram negative bacilli; pleomorphic	? Sluggish	Bright pink, lenticular; deep	–	–
			D2a/3	I	2a	Gram negative bacilli; pleomorphic	Motile	Very small colorless; deep	–	–
	B. corallinus	Motile Gel. not liq. Milk alk. Pink pig. Nitrates red.	B 10/7	I	10	Gram negative bacilli	? Sluggish	Small, round, bright pink: deep	–	±§
C. Yellow chromogenic bacilli Types—B. radiatus ochraceus arborescens		Yellow pig.	B36/5	I	36 (37.5°C.)	Gram negative bacilli: bipolar		Yellow lenticular; deep	–	–
		Not studied biologically	B12/3†	I	12	Bipolar		Yellow lenticular; deep		
			A31/4‡		31	Gram negative bacilli; small		Yellow lenticular; deep		
			A31/6‡		31	Gram negative bacilli; pleomorphic		Yellow lenticular; deep		
2. Unidentified			OCIII*	I		Gram positive bacilli large, shaggy	–		–	–

3. Group XIV: Chromogenic micrococci		Gram + Non-motile Gel. not liq. Milk unchanged Nitrates not red. Pink pig.	B 8/6	I	8	Gram positive cocci: single, pairs, clusters	–	Deep pink: surface	–	–
4. Group XI: Bacilli do not liq. gel. and do cause alkalinity in milk Type—B. pinnatus solitarius etc.	B. rugosum pinnatus solitarius	Motile Gel. not liq. Milk alk., pep. Nitrates red.	B12–2/6	II	12–2	Gram negative bacilli; small, pleomorphic	? Sluggish	Mucoid, translucent	–	+
			A12–1/8	II	12–1	Gram negative bacilli; small, pleomorphic	Motile	Dull, pink, irregular; deep	–	+
	B. candicans	Non-motile Gel. not. liq. Milk alk. Nitrates not red. or reduced	B12–2/4	II	12–2	Gram negative bacilli; small, pleomorphic	–	Large, white, opaque, ringed pin-point nucleus; deep	–	–
	refractum refractum		A12–1/9	I	12–1	Gram negative bacilli; slender, tend. thread forms	–	Small, fluorescent, round; deep	–	+
			B36R/4	II	36 (20°C.)	Gram negative bacilli; small	–	Large, pale, translucent; deep	–	+
	tiogense		B12/8	II	12	Gram negative bacilli; small	–	Dull pink, irregular; deep	–	+
5. Group X: Bacilli do not liq. gel. but do acidify milk Type—B. punctatus lacticum etc.	B. lacticum refractum tiogense	Non-motile Gel. not liq. Milk acid, pep., sec. alk. Nitrates not red.	B36/4	IV	36 (37.5°C.)	Gram negative bacilli; pleomorphic	–	Round, cream white; deep	–	–
	B. formicum	Sluggish Gel. not liq. Milk acid, coag. Nitrates red. or not reduced	B10/8	III	10	Gram negative bacilli; small	? Sluggish	White, translucent, ringed, pin-point nucleus	–	+

CLASSIFICATION ACCORDING TO JORDAN	SOMEWHAT SIMILAR TO ORGANISM	GROUP PROPERTIES	STRAINS ISOLATED	FEVER GROUP	NUMBER OF H_2O SOURCE	MORPHOLOGY	MOTILITY	AGAR COLONIES	INDOLE	NITRATES REDUCED
5. Group X: Bacilli do not liq. gel. but do acidify milk Type—B. punctatus lacticum etc.	B. formicum	Sluggish Gel. not liq. Milk acid, coag. Nitrates red. or not reduced	B23B/4	III	23 before	Gram negative bacilli; small	? Sluggish	Small, round, glistening	−	+
			B20/4	III	23 before	Gram negative bacilli; small	? Sluggish	Small lenticular, cream white; deep	−	+
			B23B/1	III	23 before	Gram negative bacilli; small	? Sluggish	White, opaque, hazy edge	−	−
			B20/1	III	20	Gram negative bacilli; small	? Sluggish	Fluorescent	−	+
			B23A/1	III	23 after	Gram negative bacilli; pleomorphic	? Sluggish	Large, white, opaque, ringed; pin-point nucleus; deep	−	+
			A31/1	III	31	Gram negative bacilli; small	? Sluggish	Large, white, round opaque; deep	−	−
			A31/5	III or IV	31	Gram negative bacilli; slender	? Sluggish	Large, translucent, ringed, flat; deep	−	−
			OTI *	III		Gram negative bacilli; small	−		−	+

Note: Fever group:
I = no fever
II = mild fever
III = severe fever
IV = death
} As a result of intravenous injections of extracts.

* Strains carried over from previous year's work.
† Not carried through series of tests.
‡ Not carried through series of tests nor tested in animals as to fever production.
§ Indefinite; negative at one time, positive at another.

distilled water seeded with this strain exhibited viable organisms seventy days subsequent to inoculation. On the other hand, it was very difficult to secure viable transfers from a plain agar slant one month old. Furthermore, no growth at all could be obtained on real infusion blood agar slants.

DISCUSSION OF RESULTS

A cultural study of twenty-five strains of bacteria isolated from twelve distilled waters, together with a consideration of the physiological reactions produced in rabbits by injection of their aqueous extracts (reported in Part I), establishes the following correlations:

1. All the strains of bacteria which are not responsible for the production of pyrogen in distilled water, with the exception of one, appear to fall within the two following groups described by Jordan (2) in his classification of River Water Bacteria: namely, group XIII—A. Red chromogenic bacilli, typified by B. ruber; and C. Yellow chromogenic bacilli, typified by B. radiatus, ochraceus and arborescens. Group XIV—Chromogenic micrococci,—this group is represented by only one organism—B8/6—in the present study.

2. The strains of bacteria which are responsible for the production of mild fever seem to be related to Jordan's group XI, which are bacilli characterized by non-liquefaction of gelatin and a production of alkalinity in milk. They are typified by B. pinnatus, solitarius candicans, refractum and tiogense.

3. The strains of bacteria responsible for the production of severe fever and even death, as in case of B36/4 and A31/1, all seem to fall within the group X described by Jordan. As he states, they are somewhat similar to the Coli group except no gas is produced in sugar broth and no indole is formed. They are characterized chiefly by non-liquefaction of gelatin and the production of acid in milk, and are typified by B. punctatus, lacticum, formicum, etc. Furthermore, these strains of bacteria, according to the classification of the Committee of the Society of American Bacteriologists could represent an extra unnamed tribe of the Bacteriaceae, belonging somewhere between the Erwineae and the Bactereae.

4. None of the strains isolated fill the exact description of the type organisms named, but differ only in such minor respects that it is safe to say that they can be placed in the same group.

We wish to express our appreciation to Prof. H. Gideon Wells for making these studies possible and to Prof. E. O. Jordan for helpful criticisms in the classification of the bacteria.

BIBLIOGRAPHY

(1) PRESCOTT AND WINSLOW: Elements of water bacteriology, 1915.
(2) JORDON: Journ. Hyg., 1903, iii, 1.
(3) WINSLOW ET AL: Journ. Bact., 1920, v, 191.

THE METABOLISM IN PREGNANCY

I. Changes in the Tension of Alveolar Carbon Dioxide[1]

A. W. ROWE, H. L. BANKS AND M. D. ALCOTT

From the Department of Chemistry, Evans Memorial, Boston, Mass.

Received for publication October 3, 1924

In a study of the general metabolism of normal pregnancy begun some years ago, one of the first points presenting for investigation was the lowered tension of the alveolar carbon dioxide shown by the non-toxic pregnant female. That an appreciable fall below the normal level was an incident of the condition was investigated some years ago by Hasselbalch and Gammeltoft (1) and had been earlier observed by Hasselbalch (2) and by Leimdörfer, Novak and Porges (3). The former used the Haldane method (4), which approximates the tension of the arterial blood; while the latter, using the Plesch method of collection (5) secured results more nearly in harmony with venous tensions. In addition, several more recent papers have observed lowered tension as an incident to other studies. While a general tendency was manifest throughout, the lack of uniformity of the conditions of observation made the combined data difficult of collation. For this reason, the present study was undertaken to establish a base line of normal performance. Certain supplementary urine studies were also undertaken in the hope of clarifying the cause of the acidotic state.

Outline of study: *a. Subjects.* As the study had certain clinical aspects not touched on in this paper, subjects for this study were drawn from two divergent sources. Group I was composed of cases drawn from the prenatal sevice of a large metropolitan hospital, supplemented by a few volunteers drawn from private practice. Group II was composed of two separate groups (A and B) of young women resident in a home for unmarried mothers. The first group represented the course of normal pregnancies uncontrolled except by the conditions of the home; the second, women who were maintained throughout the period of study under standard uniform conditions of daily regimen. The pertinent data are compiled as averages in table 1.

Only normal cases are reported here. The urines were carefully followed for the appearance of acetone, and a positive test excluded the subject

[1] Presented at the annual meeting of the American Society of Biological Chemists, Toronto, December 29, 1923.

from the study. The development of any other pathology was equally ground for rejection.

b. Collections. In groups I and II A, observations were made at two-week intervals, and every week in group II B. Urines were collected over toluene for the 24-hour period prior to each test. The alveolar CO_2 was taken in the morning with the patient in basal condition (i.e., at rest and in a post-absorptive state).

c. Methods. The ammonia and the titratable acidity of the urines were determined by the Folin methods (6), (7) the phosphate titrated by the usual uranium acetate procedure.

To standardize the CO_2 measurements, certain preliminary determinations were carried out. With the large number of observations desired it seemed best to utilize one of the two existing methods which have obtained a somewhat general use; namely, the procedures suggested

TABLE I

Physical data

SERIES	NUMBER	AGE	HEIGHT	WEIGHT AT DELIVERY
		years	*cm.*	*kgm.*
I	25	28	158	67.3
II A	21	18	160	64.9
II B	22	20	161	55.5

respectively by Fredericia (8) and by Marriott (9). The first approximates arterial, the second venous tensions.

1. Respiratory phase. The Fredericia method collects a portion of a single expiration taken during a normal expulsive effort and without preliminary forced inspiration. It is obvious that the composition of the expired air will vary somewhat, depending upon that portion of the respiratory phase collected. The procedure adopted was to have the patient begin breathing through the instrument at the moment of inception of expiration, and to continue until all of the tidal and a portion of the supplemental air has passed through the collecting chamber before sealing off the sample. In this way the initial pure air content of the apparatus is displaced by air containing an increasing CO_2 content, which tends to lessen the error of dilution of the sample. A sample test (20 observations) of the efficacy of this procedure showed a mean value of 42.1 mm. $\pm$ 2.0 mm. and an average of 41.9 mm.

2. Indirect comparison of Haldane and Fredericia. A Fredericia apparatus was modified by substituting a three-way cock with one capillary outlet for the usual two-way intake cock. A sample of air was collected from the patient and then transferred to the Haldane apparatus by

mercury displacement. This allowed the analysis of the middle portion of the trapped sample, and eliminated possible dilution of the alveolar air during transference. A second sample was at once collected in the Fredericia tube, and both samples analyzed. The variations observed were of the order of those noted in the duplication of the respiratory phase as shown in table 2.

TABLE 2

CO_2 by Haldane—Fredericia

Indirect

Average + deviation	(8 cases)	+1.9 mm.
Average − deviation	(13 cases)	−1.7 mm.
Average deviation	(21 cases)	−0.3 mm.

3. Direct comparison of Haldane and Fredericia. A sample of air was collected in a modified Plesch-Higgins (10) container.[2] Samples from the container were transferred by negative mercury displacement to both the Fredericia and Haldane apparatus, and analyzed. The results with 15 cases showed the relative dependability of the Fredericia apparatus.

TABLE 3

CO_2 by Haldane-Fredericia

Direct

Average + deviation	(8 cases)	+1.1 mm.
Average − deviation	(5 cases)	−1.9 mm.
No deviation	(2 cases)	
Average deviation	(15 cases)	±0

4. Comparison of Marriott and Fredericia. It was the original intention to make duplicate determinations on each case by both the Fredericia and Marriott methods. Preliminary observations showed that no striking differences were observed under the conditions of experiment. Further, the more elaborate precautions necessary for the sterilization of the latter apparatus, coupled with a psychological inhibition shown by many of the patients, led to its discontinuance.

RESULTS. Under the conditions imposed by the character of the subjects, certain differences existed in the respective cycles studied. The members of group I came to the hospital for delivery and were discharged

[2] The modification consisted in substituting a spun aluminium cup for the heavy metal base and replacing the heavy three-way metal gate valve with large glass and thick-walled rubber connections. A marked saving in weight and increase in convenience was thus secured.

to their homes within a short time (usually two weeks) of their confinement. Measurements could be begun, however, fairly early in the pregnancy. With group II, on the other hand, they first became available when admitted to the home, an average of between two and three months before delivery. After confinement, however, they were retained in the home until their economic status could be adjusted. It was possible therefore

TABLE 4

Acid elimination

PERIOD	CO_2 TENSION			P_2O_5 PER 24 HOURS			TOTAL ACID (EQUIV. CC. $\frac{N}{10}$ ACID)		
	Group I	Group II	Total average	Group I	Group II	Total average	Group I	Group II	Total average
weeks	*mm. Hg*	*mm. Hg*	*mm. Hg*	*grams*	*grams*	*grams*			
30–29	(24)		(24)						
28–27	(23)		(23)						
26–25	(27)		(27)	2.14		2.14	560		560
24–23	32		32	1.69		1.69	610		610
22–21	32		32	2.07		2.07	580		580
20–19	30		30	1.72		1.72	710		710
18–17	30		30	2.10		2.10	570		570
16–15	31		31	1.51		1.51	460		460
14–13	31		31	1.71	1.90	1.72	530		530
12–11	29	35	31	1.68	1.98	1.74	640		640
10–9	33	33	33	1.54	1.87	1.65	490	640	550
8–7	30	32	32	1.62	1.67	1.64	660	690	680
6–5	32	31	32	1.53	1.98	1.76	520	710	630
4–3	31	32	31	1.86	2.11	1.99	680	680	680
2–1	37	31	32	1.90	1.93	1.92	610	610	610
Delivered									
1–2	36	31	33		2.16	2.16		890	890
3–4		31	31		2.35	2.35		750	750
5–6		34	34		2.39	2.39		520	520
7–8		36	36						
9–10		(35)	(35)		1.78	1.78		600	600

to continue the studies made with them for a period almost equal to the ante-partum interval.

For the sake of brevity the results obtained are presented as averages based upon the time interval of observation. All the significant data are gathered in the above table. The acid values correspond to the total acid elimination, this being the sum of the titratable acidity plus the acid equivalent of the ammonia elimination. This convention was used by Fitz and Van Slyke (11) in another connection.

Values for the several factors which are enclosed in brackets, indicated averages drawn from a number of observations, inadequate to confer full authority. Total averages are compiled from group averages by weighing each according to the number of observations included.

a. Carbon dioxide. The presentation of data by the compilation of averages tends to mask individual variations from the base line. On

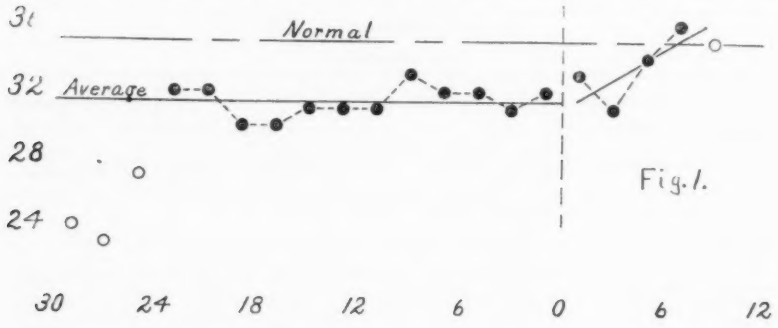


Fig. 1.

Fig. 2.

Fig. 1. Alveolar carbon dioxide tensions. Millimeters of mercury / time in weeks.

Fig. 2. Acid elimination. Equivalent cubic centimeters of N/10 acid / time in weeks.

the other hand, if the average be based upon an adequate number of observations, it has an authority in indicating tendencies of which individual measurements are devoid. Considering the diversity of uncontrollable extraneous factors which must obtrude in a study like the present, the method of averages seems to be the sole means of approach. The variation of the CO_2 tensions is strikingly shown by reducing them to graphic representation (fig. 1).

The average level throughout the ante-partum period is 31.4 mm., a value indicating a mild degree of acid intoxication if the conventional practice be followed of indicating 35 mm. as the low limit of normalcy. With delivery there is a slow upward trend, so that in a space of two months the tensions have returned to the inferior portion of the normal zone.

b. Phosphoric acid. The usually accepted figures for a normal phosphate elimination vary between the generous limits of 1 to 5 grams, with perhaps 2.5 grams representing the conventional average. On this basis there would seem to be a moderate degree of phophate retention. The fluctuation in successive periods—particularly noticeable from the 26th to the 14th week ante-partum, when there is an almost rhythmic sequence—never raises the level to the arbitrary value of 2.5 grams. The post-partum levels, although higher than those before delivery, do not quite attain it. The average elimination ante-partum is 1.77 grams, which is that observed during the 9th and 10th weeks post-partum. In view of the doubtful character of the arbitrary standard and the values here obtained, a significant retention of phosphoric acid is dubious.

c. Acid elimination. Here again usually accepted standards lack sharpness of definition. An average ammonia elimination on a mixed diet is given as from 0.5 to 0.7 gram, corresponding to from 300 to 400 cc. of N/10 acid. Similarly, titratable acid may vary from an equivalence to 200 to 500 cc. of N/10 acid. If these be summated, the allowable limits of normalcy assume the liberal boundaries of 500 to 900 cc. By these criteria, there is no evidence of depressed acid elimination in the values obtained in this study. In only one period does the average fall below the conventional lower limit (16th to 15th weeks) and the average ante-partum value is 600 cc., a normal value. The higher acid elimination in the first weeks following delivery assume a suggestive sequence. Far from implying a restoration to normal of a previously impaired elimination, the curve would imply additional acid formation as an incident of confinement. The last post-partum observation is identical with the mean of the ante-partum observations. This is also shown most clearly in figure 2. Here the ammonia and acid moieties of series II are plotted separately, and the combined curve of total acid elimination added for reference. While the ammonia values show some variation in the ante-partum period, they are always within normal limits, and the same is true of the titrated acid. After delivery this latter fraction shows a temporary marked increase, the ammonia remaining fairly constant and at a lower level than during the pregnancy. Within the period of the investigation, the several components have resumed their ante-partum level.

To conclude, then, these observations do not present certain evidence

of either phosphate retention or depressed acid elimination as a cause for the proven acidosis of pregnancy.

SUMMARY

The significant points of this paper may be briefly summarized as follows:

1. Data on the tension of alveolar carbon dioxide, and the elimination of acid and of phosphates of the normal pregnant woman have been secured by the observation of several series of cases.

2. The CO_2 observations indicate a mild degree of acidosis, which apparently operates throughout the period of gestation.

3. The values for acid and phosphate elimination, while not high, are substantially normal by generally accepted conventional standards.

4. Acid retention is apparently not the cause of the existing acidosis.

In conclusion, the authors take great pleasure in extending their thanks to the many subjects who coöperated with them, at no little personal inconvenience and discomfort.

Also to Doctors Ham, Smith, Ruggles and Diehl for the courteous reference of cases.

Particular indebtedness is expressed to Dr. George H. Earl, through whose kindness and personal interest the observation of the second group was made possible.

BIBLIOGRAPHY

(1) HASSELBALCH AND GAMMELTOFT: Biochem. Zeitschr., 1915, lxviii, 20ℓ.
(2) HASSELBALCH: Skand. Arch. Physiol., 1912, xxvii.
(3) LEIMDÖRFER, NOVAK AND PORGES: Zeitschr. klin. Med., 1912, lxxv.
(4) HALDANE: Journ. Physiol., 1905, xxxii.
(5) PLESCH: Zeitschr. exper. Path. u. Therap., 1909, vi, 380.
(6) FOLIN AND MACALLUM: Journ. Biol. Chem., 1912, xi, 523.
(7) FOLIN: This Journal, 1903, ix, 265; 1905, xiii, 102.
(8) FREDERICIA: Hospital stidende, 1914, lvii, 585.
(9) MARRIOTT: Journ. Amer. Med. Assoc., 1916, lxvi, 1594.
(10) Carnegie Inst. Pub. 203, 1915, 168.
(11) FITZ AND VAN SLYKE: Journ. Biol. Chem., 1917, xxx, 389.

THE METABOLISM IN PREGNANCY

II. Changes in the Basal Metabolic Rate

A. W. ROWE, M. D. ALCOTT AND E. MORTIMER[1]

From the Department of Chemistry, Evans Memorial, Boston, Mass.

Received for publication October 3, 1924

Observations of the basal metabolic rate of the normal pregnant woman are surprisingly limited in number and extent when one considers the widespread interest in this important biometric factor. Records exist of single cases studied throughout the gestation period by Magnus Levy (1), Hasselbalch (2), and most recently Root and Root (3). Incomplete records were made on three cases by Zuntz (4) and shortly after Carpenter and Murlin (5) reported a most comprehensive and complete study, but the periods of observation averaged only two weeks ante- and the same post-partum. In addition to these, two clinical studies have recently appeared reported by Baer (6) and by Cornell (7).[2] The brief period of study, the paucity of observations on a single case (an average of less than three), the inclusion of pathological cases, and most significant, the existence of a potent psychic element (recognized by the second author) nullifies in largest measure the significance of the data. The inevitable element of individual variation is manifest in the records of the nine cases

[1] Presented before the American Society of Biological Chemists, St. Louis, December 29, 1923.

[2] Since this paper was presented, an article by Mahnert (8) has appeared, dealing with metabolic changes in pregnancy. The only data pertinent to the present discussion give the results of four observations on two women just prior to confinement, one of whom shows an increase of 7 per cent, the other a loss of 14 per cent in 24 and 15 days respectively. While complete data are lacking, the first measurement on the second case shows a rate somewhere between 30 per cent and 40 per cent above the Harris-Benedict normal. It would seem possible that a psychological element existed, for which no allowance has been made. Twelve cases in thirty show an average loss of 1 kgm. in the three days immediately preceding confinement. No data are given concerning the other eighteen, who presumably showed no change, or a continual increase.

Through the courtesy of Dr. Walter M. Boothby, the writers have been privileged to read two papers in manuscript by Sandiford, and Sandiford and Wheeler respectively, based upon the very complete study of a single case at the Mayo Clinic. The authors by a series of interesting assumptions, arrive at conclusions somewhat at variance with those presented here. As the papers will be published shortly, discussion may suitably be postponed.

cited above. To establish a base line which would permit of relative comparison and define allowable limits of variation, a long series of observations made under standardized conditions and on an appreciable number of cases are essential. Recognizing this principle, the present authors several years ago undertook to compile the necessary data, and the present paper embodies certain of the results obtained. Methods and results can be discussed without further introduction.

SUBJECTS. As the present study has certain clinical aspects, not touched upon in this presentation, subjects were drawn from two independent and widely different sources. The first group was recruited from the prenatal service of a large metropolitan hospital supplemented by a few private cases, who volunteered for the study and were referred by colleagues. This group comprised women living in their own homes under no dietary or other special control; in other words, women whose pregnancies were passed under the conditions of their normal habit of life.

The second group was taken from among the inmates of a nursing home for unmarried mothers. Here the conditions were diametrically opposite; the patients being on a standardized dietary and each with a stated round of duties to be performed—in short, following a regimen wholly foreign to their normal course, and living under uniform standardized conditions.

In addition, with this second group, the conditions of the pregnancy introduced a psychic element of which the first was devoid.

Only normal healthy individuals were selected, and only those retained in the study who maintained this condition throughout. The elimination of those who developed apparent abnormality, coupled with the defection of volunteers whose interest waned—a far larger proportion—circumscribed the number of completed cases in a most material manner. The present report is based upon 46 cases which completed the test, the number starting being well in excess of 100.

SCHEDULE AND METHODS. With group I all measurements were made at the laboratory; with group II, at the Home where they were resident. The same general schedule was followed with both.

During the 24 hours preceding the test, the total urine was collected, using toluene as a preservative. On the day of the test the fasting patient came to the metabolism room, was placed comfortably, with loosened clothing, on the metabolism couch and remained in a state of quiescence —never less than thirty minutes—until repeated pulse readings gave evidence of a basal condition. The basal metabolism was then determined, two or possibly three independent tests of ten minutes each being made. In our experience, the latter number is the maximum which it is expedient to carry out at any one time. Nervousness and restlessness destroy the accuracy of the determination, and both these factors manifest themselves if the test be too protracted. In this connection, it is of interest that the

first day's tests were usually worthless because of nervousness and hence were discarded—the second, in the majority of cases, gave wholly reliable results and after several days' repeated experience the patients relaxed to a point of somnolence, which equally had to be combatted.

Frequent pulse, temperature and blood pressure records were made on each day. The basal rate measured, the patient then recorded the lung capacity, a sample of alveolar air was secured, and finally, in an adjacent room, the nude weight and the complementary vital capacity measurements secured. The entire test usually took something over 90 minutes.

Tests were carried out on the individual cases at two-week intervals, a day being selected at the beginning of the test which would best conform to the patient's convenience.

Methods. The basal rate measurements were made with Benedict portable respiration units. Every precaution was observed to secure mechanical accuracy. With the first group two instruments were used interchangeably, with group II a single instrument was kept at the Home throughout the course of the studies.

Lung capacities were determined with a water sealed spirometer with automatic recording device. Individual glass mouth pieces of large bore and varying size were designed for the study.

Circumferential measurements were made with steel tapes, the standing and trunk height with calibrated rods of standard pattern. In determining the trunk height, difficulty was experienced in persuading the patients to assume the attitude seated on the floor recommended by Dreyer (9). Compliance was secured by fixing securely the measuring rod to the back of a stool 13 inches high. The buttocks were pressed against the back board and the knees slightly elevated above the horizontal, and the lower leg vertical. In this way the gluteal variation was reduced to a minimum. The constancy of the measurements made in this manner was most satisfactory. Blood pressures were taken by auscultation, using a soft cuff and mercury column instrument. Oral temperatures were taken with standardized mercury clinical thermometers. This was not an ideal procedure but was conditioned, as were other compromises, by the necessity of maintaining the coöperation of the subjects.

Following the practice of this laboratory, comparisons of the observed metabolic rate were made with both the Harris-Benedict and the AubduBois standards. The average of the two comparisons was taken as the indicated variation. As practically all of the patients approximated normal body configuration, the correlation of the two sets of values was excellent. Divergencies were observed with some of the younger patients in group II, but as relative values were desired, no error of appreciable magnitude was introduced.

Results. To present the large number of data in detail would be pro-

TABLE 1

Series I, case 28

Name: R., Mrs. E. Height: 175 cm. Trunk: 85 cm. Age: 30 years.
Weight of child: 2.5 kgm.
Previous conceptions: 1. Living: 0. Dead: 1. Miscarriage: 1.
Details of labor: Easy L. O. A. delivery, no lacerations

	WEEK										
	22	20	13	11	9	7	5	3	1		1
Blood pressure (systolic)	140	118	108	100	96	102	102	120	106		
Blood pressure (diastolic)	70	62	56	62	58	70	64	64	68		
Temperature	97.4	97.4	97.4	97.6	96.5	98.4	97.4	97.9	97.4		98.6
Pulse (r.p.m.)	100-80	92-78	81	76	80	78	76	70	65		68
Respiration (r.p.m.)	27	30	28	28	26	19	26	22	26		20
Weight (kgm.)	58.3	58.7	60.3	62.4	63.7	63.9	64.2	65.8	66.8		63.0
Area (sq. m.)	1.71	1.72	1.74	1.76	1.78	1.78	1.78	1.80	1.81		1.77
Chest (cm.)	75	75.5	76.5	78	75.5	80	83	82	84		
Oxygen consumption (cc. p.m.)	176.1	177.5	189.5	197.7	215.1	200.5	201.0	209.8	210.7		211.3
Basal metabolism (observed)	1206	1235	1317	1374	1494	1393	1397	1458	1447		1468
Mean per cent	−17	−15	−11	−8	±0	−8	−8	−5	−6		−2
Vital capacity (cc.)	2800	2300	1900	2500	1900	1800	2140	2040	2200		

Delivery

TABLE 2

Series II, case 13

Name: M—. Height: 158 cm. Age: 19 years.
Weight of child: 3.09 kgm.
Previous conceptions: 0.
Details of labor: Long and tedious. Forceps at brim.

	WEEK									
	13	11	9	7	4	3	1	0	1	3
Blood pressure (systolic)	114	108.	108	112	114	114	110		106	120
Blood pressure (diastolic)	64	62.	64	60	74	70	72		58	80
Temperature	98.2	97.2	96.6	97.8	97.6	98.0	97.8		98.6	97.0
Pulse (r.p.m.)	62	64.	66	68	67	91	72		57	56
Respiration (r.p.m.)	17	17	20	18	18	20	21		19	18
Weight (kgm.)	59.4	60.7	60.8	61.7	61.9	64.5	65.0		58.0	57.0
Area (sq. m.)	1.60	1.61	1.61	1.62	1.63	1.66	1.67	Delivered	1.59	1.57
Oxygen consumption (cc. p.m.)	187.3	179.6	185.2	182.2	187.7	208.8	200.3		202.2	185.4
Basal metabolism (observed)	1301	1248	1287	1266	1304	1451	1392		1405	1288
Mean deviation (per cent)	−10	−14	−12	−14	−11	−3	−8		−2	−9

TABLE 3

CASE NUMBER	PARA	AGE	BEFORE DELIVERY			AFTER DELIVERY				REMARKS
			Weeks observed	Average gain per week	Final weight (calculated)	Day observed	Weight loss	Per cent	Child	
Series I										
				kilos						
1	1	33	16	0.43	66.6	12	15.1	23	4.04	
2	2	30	11	0.40	70.4	7	6.7	10	2.41	
3	1	22	19	0.52	53.9	16	10.8	20	3.05	
4	1	18	20	0.39	68.0	11	9.0	13	3.23	
5	1	23	22	0.36	54.8	5	9.8	18	2.86	
6	5	30	19	0.28	56.9	11	10.0	18	2.73	
7	2	26	25	0.35	73.6				2.73	Post-partum weight not obtainable
8	3	22	14	0.49	79.0	14	10.8	14	3.27	
9	4	32	30	0.31	61.1	6	9.1	15	3.18	
10	3	28	26	0.33	70.3	7	10.3	15	3.82	
11	5	36	16	(−0.12)	68.6	4	8.6	12	3.23	Showed progressive loss of weight without evident pathology
12	2	35	27	0.23	67.9				4.13	Developed puerperal septicemia
13	2	30	21	0.33	66.3	9	8.3	13	2.55	
14	1	19	13	0.74	70.8				3.86	Delivered out of town
15	1	32	20	0.28	76.0	10	12.3	16	4.04	
16	2	27	29	0.48	56.9				3.96	Post-partum observations 13 weeks after delivery
17	2	25	24	0.38	85.2	8	9.2	11	2.86	
18	3	37	21	0.42	80.0	11	10.0	13	3.09	
19	1	22	14	0.50	68.0	5	11.0	18	3.45	
20	2	30	22	0.40	67.2	11	4.2	6	2.55	
21	1	21	24	0.23	71.5				4.32	Post-partum weight not obtainable
22	3	37	34	0.24					2.95	Extrapolation uncertain. Patient not seen for many weeks
23	3	31	18	0.30	58.9	6	4.2	7	3.14	
24	3	32	11	0.21	71.1				3.77	Post-partum weight not obtainable
25	1	28	19	0.15	58.6				3.41	Post-partum weight not obtainable
High.....		37	34	0.74	85.2	16	15.1	23	4 32	
Low.....		18	11	0.15	53.9	4	4.2	6	2.41	
Average..		28	21	0.32	67.3	9	9.4	14	3.31	

TABLE 3—*Concluded*

CASE NUMBER	PARA	AGE	BEFORE DELIVERY			AFTER DELIVERY				REMARKS
			Weeks observed	Average gain per week	Final weight (calculated)	Day observed	Weight loss	Per cent	Child	
					Series II					
				kilos						
1	1	17	9	0.36	67.2	10	10.7	16	3.45	
2	1	34	11	0.34	57 7	10	6.0	10	3.09	
3	1	16	7	0.67	74.2	11	10.5	14	3.77	
4	1	15	5	0.45	64.2	20	12.0	19	3.68	
5	1	21	12	1.03	74.7	5	13.2	18	2.95	
6	1	18	7	0.28	69.3	9	7.5	11	3.41	
7	1	17	11	0.37	67.0	6	9.0	13	3.45	
8	1	21	12	0.32	48.9	17	7.7	16	2.91	
9	1	22	4	0.85	69.4	14	4.8	7	2.50	
10	1	13	7	0.40	55.7	7	9.0	16	3.68	
11	1	19	13	0.47	65.5	14	8.5	13	3.09	
12	1	20	11	0.66	67 5	11	10 2	15	2.86	
13	1	14	6	0.02	49.7	12	9.3	19	2.77	
14	1	22	10	0.11	81.7	7	7.1	9	3.77	
15	1	14	9	0.81	61.5	11	7.1	12	2.95	
16	1	17	9	0.79	75.7	12	14.2	19	4.09	
17	1	18	9	0.83	64.4	8	9.8	15	3.59	
18	1	17	5						3.00	Extrapolation uncertain
19	1	21	7	0.73	71.9	12	10.5	15	3.08	
20	1	15	6	0.44	57.2	12	5.6	10	2.00	
21	1	40	7	0.43	55.5				3.04	Died 42 hours after cesarean section
High.....		40	13	1.03	81.7	20	14.2	19	4.09	
Low.....		13	4	0.02	48.9	5	4.8	7	2.00	
Average...		18	8	0.52	64.9	11	9.1	14	3.20	

hibitively space-consuming. Further, the object of the study was to secure enough observations to permit the merging of individual variations in a representative average. Two sample protocols may be given, however, to illustrate individual conformity to the resulting generalization. All records date forward and back from the day of confinement, as this represents the fixed point in the cycle. In calculating averages, observations have been grouped in two-week intervals. As the greater number of the cases were measured on this basis, this procedure insures the reappearance of each case at appropriate points in the general curve, which in summation they define.

a. Weight. The data for the weight changes can best be presented in tabular form, utilizing the opportunity to present certain general descriptive facts about each subject. These records are collated in table 3.

It will be noted that two-thirds of group I are multiparae, while all of II had conceived for the first time. The character of the patients determined this difference, as well as the low age average in the second group. Case 11 was only 12 years of age at the time of impregnation and over one-fourth of the group was 16 years or less.

Group I shows an average weekly increase of 0.32 kilo, while the second group gave the much higher factor of 0.52. Several elements undoubtedly contributed to cause this difference, among which may be lack of exercise and standard liberal dietary without economic curtailment. Inspection of individual records would seem to eliminate age per se as an influence. The final weights are calculated from the individual coefficients to provide a uniform base line for subsequent comparisons. In no case did the extrapolation affect the result by more than 2 per cent, and in the majority of cases it was less than 1 per cent. The loss of weight on delivery shows a surprising agreement between the two averages. This correlation is also exhibited in the weights of the children. When one considers the widely divergent conditions under which the two sets of pregnancies were conducted, the observed agreements enforce confidence in the magnitude of the coefficients obtained.

b. Basal metabolism. As before stated, the basal metabolic rate was determined directly as the mean of two or more concordant measurements, and the result compared both with the Harris-Benedict and Aub-duBois standards. The mean of these comparisons was taken as the observed deviation from the calculated normal. The observed changes can best be presented in graphic form. Each point on the curve represents the average of all observations made within a specific two-week interval. Points are plotted on the odd week ordinate as representing the mean position of any two-week period. Where a limited number of data were available the point is represented as a hollow circle, where truly representative as a solid dot. The results with series I and II are given respectively in figure 1. A large scale was purposely adopted to emphasize deviations from the characteristic curve.

In both series it will be seen that the change is linear during the antepartum period, and that the slope is practically identical. Expressed as per cent, the change in group I is +0.53 per cent per week—in series II 0.60 per cent.

Fig. 1. Deviation of observed basal rate from calculated normal. Per cent / time in weeks.

Fig. 2. Observed energy consumption. Calculated as basal rate for 24 hours. Calories per 24 hours / time in weeks.

Fig. 3. Lung capacities. Liters / time in weeks.

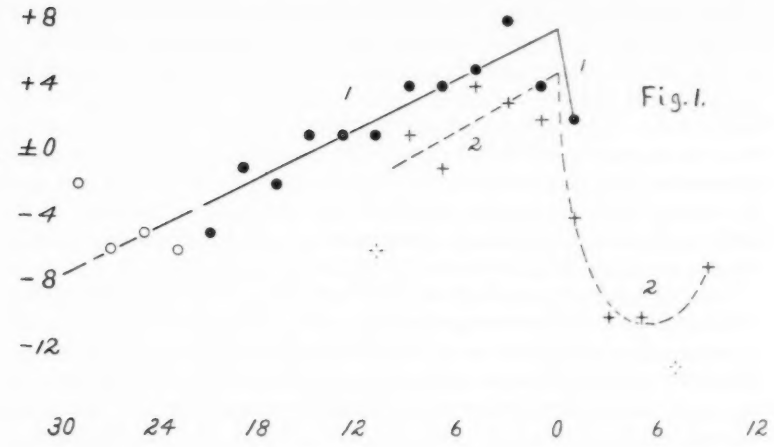


Fig. 1.

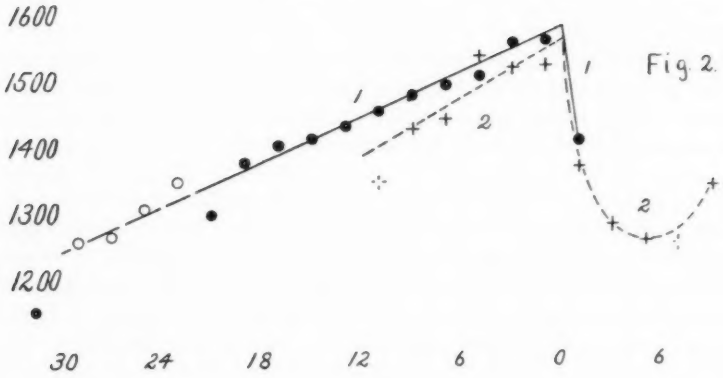


Fig. 2.

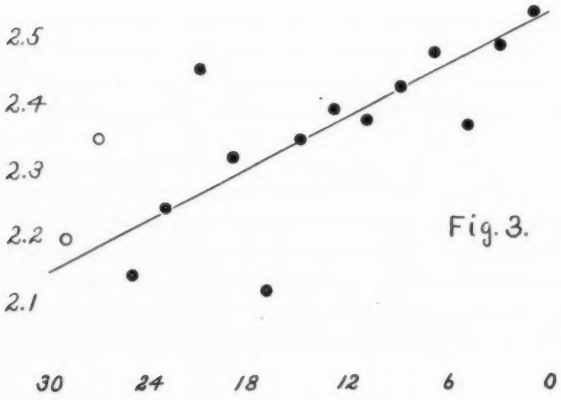


Fig. 3.

The post-partum portions of the curves are decidedly different. With the first series, the patients came to the hospital for delivery; and one, or at most, two, measurements were made before their discharge. With the second group, on the other hand, after delivery they remained in the Home until their economic status could be adjusted, usually a period of two months or more: in this way it was possible to secure the repeated observations which define the second phase of curve II. A definite and protracted fall is observed which persists during the first month. A minimum is then reached, and slowly the curve turns upward. It is still 7 per cent below the base line at the expiration of nine weeks.

It is a matter of some interest that both curves remain within the so-called allowable limits of variation from the normal base line. True, the post-partum curve of the second series touches the inferior limit of this arbitrary standard, but the variations fall inside of the conventional ± 10 per cent. It is, naturally, fortuitous that the curve of series I crosses the base line midway in the 30-week interval. Curve II passes this point in the eighth week before delivery, and yet lies but 3 per cent below the curve of the first series.

Another method of presenting the data is to record the actual metabolic rate as observed without the interjection of any arbitrary standard of comparison. This method takes no account of the increase in body weight, a recognized factor in the determination of the magnitude. The data for the two series presented in this wise are given in figure 2. In series I the rise is from 1245 to 1580 calories in a period of 30 weeks. This corresponds to an increment of $+0.90$ per cent per week. Similarly, the curve of series II progresses from 1390 to 1560 calories in a period of 12 weeks. This equals an increment of $+1.02$ per cent per week. The agreement of the absolute levels at confinement of the two series is gratifyingly concordant, although so close an agreement must be regarded as fortuitous. The mutual cancellation of errors in opposing senses operates in as large a series as the present.

The differences between the two sets of coefficients derived from the several curves may be used as a check on the correlation of the two series. These differences represent the changes produced by the variable body weight and surface area (intrinsically a function of the former). In series I this magnitude equals 0.90—0.53 or 0.37 per cent; in series II, 1.02—0.60 or 0.42 per cent. In other words, in a change approximating 1 per cent a week, 0.4 per cent is referable to increased weight. The residual and larger moiety must be ascribed to some other metabolic activity implicit in the patient's state. That it cannot be allocated solely to the additional activity of the foetus is evident from the linear character of the curve. Thirty weeks before birth, the amount of foetal protoplasm then in being, could not condition the increased energy requirement.

In the opinion of the authors, these observations offer definite support to Benedict's concept of the determination of the basal metabolic rate by the mass of active body protoplasm.

c. Vital capacity. The term "vital capacity" is defined by Dreyer (loc. cit.) as "the maximum amount of air an individual is able to expel from the lungs by voluntary effort after taking the deepest possible inspiration." He establishes three criteria of normalcy based respectively upon weight, trunk length and chest circumference, and averages the results thus obtained by comparison to secure the relation of the individual to the norm. Dreyer's standards were evidently based upon average people, and the application of his criteria to the obese or emaciated lead to results of frank absurdity. West (10) has also established criteria based respectively upon standing height and on surface area. Comparisons with these standards give far more reasonable results, although with the very obese the weight element may manifest itself unduly in the second comparison. For these reasons, and in the interest of simplicity, no comparisons have been instituted, and lung capacities recorded only as read.

There is yet another source of potential error in this magnitude, and one which may easily assume disturbing proportions. In basal metabolism measurements the subject coöperates through her passivity, while in lung capacity observations an active participation is required. The traditional nervous imbalance of this interesting condition obtrudes itself when initiative is required.

On certain days, the subject will endeavor to the utmost to give dependable maxima, on others the effort is burdensome and the results short of the truth. This factor led to the discontinuance of lung capacity measurements with the second group, and casts some doubt on the absolute values obtained with the first. The averages, obtained by the method used for the metabolism data, can best be presented in graphic form, and are assembled in curve 3.

The slope of this curve lacks something of the authority of those obtained in the basal rate measurements. The general upward trend, however, is unmistakable. Reduced to concrete arithmetical expression the rate of increase approximates +0.6 per cent per week, a change of the same order of magnitude as that of that moiety of the basal rate not conditioned by weight increase.

SUMMARY

The data presented in this paper can best be summarized in tabular form. This permits the inclusion of certain observations not touched upon in the text. Averages only are given. Certain of the observations of series II are separated into ante- and post-partum groups. In series I only the ante-partum observations are recorded.

TABLE 4
Summary

	SERIES I	SERIES II	
	Ante-partum	Ante-partum	Post-partum
Number of subjects	26	21	21
Weeks of observation	21	8	5
Blood pressure (systolic)	106	115	114
Blood pressure (diastolic)	65	76	75
Temperature	98.0	97.6	97
Pulse rate (per minute)	78	82	67
Respiration rate (per minute)	17	19	19
Age (years)	28	18	
Weight increase (per week) kilo	+0.32	+0.52	
Weight of child (kilo)	3.31	3.20	
Vital capacity increase (per week) (per cent)	+0.6		
Basal metabolism increase			
Total (per week) (per cent)	+0.90	+1.02	
Due to weight (per week) (per cent)	+0.37	+0.42	
Difference (per cent)	+0.53	+0.60	

In concluding this report the authors take great pleasure in expressing their indebtedness to the many subjects whose generous coöperation and interest made this study possible. Thanks are gladly rendered to the colleagues who referred cases, in particular to Dr. George H. Earl, through whose courtesy and personal kindness the cases of the second series were made available and finally, to the American Association for the Advancement of Science, whose generous aid materially assisted in this study.

BIBLIOGRAPHY

(1) MAGNUS-LEVY: Zeitschr. Gebürtsh. u. Gynäk., 1904, lii, 116.
(2) HASSELBALCH: Skand. Arch. Physiol., 1912, xxvii, 1.
(3) ROOT AND ROOT: Arch. Int. Med., 1923, xxxii, 411.
(4) ZUNTZ: Arch. Gynäk., 1910, xc, 452.
(5) CARPENTER AND MURLIN: Arch. Int. Med., 1911, vii, 184.
(6) BAER: Amer. Journ. Obst. and Gynec., 1921, ii, 1.
(7) CORNELL: Surg., Gynec. and Obst., 1922, xxxvi, 53.
(8) MAHNERT: Arch. Gynäk., 1924, cxxi, 620.
(9) DREYER: The assessment of physical fitness. New York, 1921.
(10) WEST: Arch. Int. Med., 1920, xv, 306.

GLOMERULAR VERSUS TUBULAR ACTIVITY IN THE MESONEPHROS OF NECTURUS: ELIMINATION OF IRON SALTS

ALDEN B. DAWSON

From Loyola University School of Medicine, Chicago

Received for publication December 9, 1924

Certain features of the anatomy of the mesonephros of Necturus make this organ favorable material for an attempt to analyze renal function.

The mesonephros is divisible into two parts, a slender anterior part, the genital kidney, and a stouter posterior part, the pelvic (secretory) kidney. In both sexes the pelvic kidney is composed of about fifty sets of renal tubules. The tubules of each set can be separated into two groups. One group consists of the so-called primary and secondary tubules which are characterized by the possession of outer segments or peritoneal canals (fig. 1). The other group consists of all the more dorsal tubules which lack outer segments and nephrostomes. The peritoneal canals of the primary and secondary tubules run from the nephrostomes on the ventral surface of the mesonephros to join these tubules in the neck region, some little distance from their renal corpuscles. The epithelium of the nephrostomes, the peritoneal canals and the necks of the tubules is covered with long slender cilia (fig. 1). The genital kidney of both sexes is composed of a single row of renal tubules. In the female, the tubules differ in no way from the primary tubules of the pelvic portion, running from renal corpuscles and possessing peritoneal canals. In the male, however, the tubules are highly modified, lack nephrostomes and assume the function of efferent ducts for the passage of sperm. (For a more complete description consult Chase (2).)

There is then in the genital kidney of the female a more or less isolated group of tubules all of which may secure materials directly either from the body cavity by way of the peritoneal canals or from the blood by way of the glomeruli. Furthermore, in the pelvic kidney of both sexes the primary and secondary tubules, though not isolated, have the same relations to body cavity and blood stream as noted for the female genital kidney. The more dorsal tubules of the pelvic kidney, however, possess no connection with the body cavity and consequently receive materials directly only from the blood. Therefore, we have here a natural preparation for the study of renal function.

It seems unnecessary in this report to restate the problem presented in renal secretion since the positions taken by the supporters of the two chief schools of thought concerning the renal mechanism is a matter of textbook information. Neither does it seem necessary to attempt any extensive survey of the enormous literature on the subject. Reference will be made only to the more recent papers which have a direct bearing on the particular aspect of the problem which is under consideration. Stieglitz (5) from his studies on the mammalian kidney concluded that iron salts were secreted only by the cells of the convoluted tubules. Furthermore, he found that iron was retained in these cells after actual secretion had ceased and that the retention of iron was cumulative with multiple injections. He, however, was working with a metanephros while our studies have been confined to a mesonephros. Recent work on mesonephroi would seem to support the belief that the mechanism of secretion in this more primitive organ differs in some respects from that of the true kidney or metanephros. In the frog, Clark (3) found that the glomeruli, when perfused through the arterial circulation, were permeable to sugar and that some sugar was reabsorbed by the tubules. Bieter and Hirschfelder (1) have also presented evidence that dyes such as phenolsulphonephthalein and sodium sulphindigotate were excreted in a dilute form through the frog's glomeruli and that the tubules were also functioning, reabsorbing the water but not secreting or reabsorbing any dye.

Fig. 1. Semidiagrammatic transverse section of pelvic kidney showing a primary and a dorsal tubule with blood supply. Arterial supply, solid black; venous drainage stippled; ciliated neck and peritoneal canal, oblique lines; proximal convoluted portion, circles; ciliated narrow segment (Henle's loop of mammalian kidney), oblique lines; distal convoluted portion, cross hatched; junctional tubule and collecting tubule, clear. (Modified after S. W. Chase (2).)

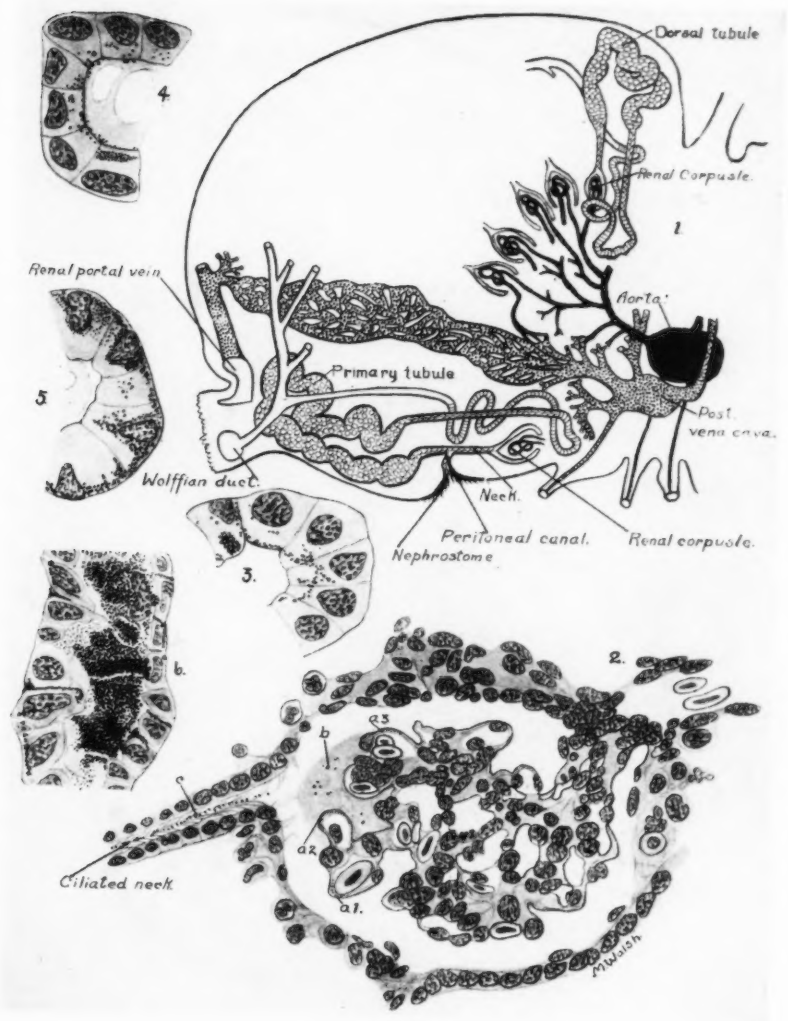
Fig. 2. Longitudinal section through a renal corpuscle and neck of the tubule. This is a composite drawing showing the several positions in which iron is found in series II, intravenous injection. Kidney tissue in wash, iron particles in black, *a1*, *a2*, *a3*, *b* and *c*.

Fig. 3. Transverse section through proximal convoluted portion of a primary tubule four hours after intraperitoneal injection (series I). Iron particles black. Note their distribution.

Fig. 4. Transverse section through proximal convoluted portion of a dorsal tubule 9 hours after intravenous injection (series II). Iron particles black. Note their distribution near the lumen.

Fig. 5. Transverse section through the proximal convoluted portion of a primary tubule after five repeated injections (series III). Iron particles black. Note their basal distribution and their concentration about the nuclei.

Fig. 6. Longitudinal section through the distal convoluted portion of a primary tubule after three repeated injections (series III). Iron particles black. Note the quantity of secretion in the lumen giving the iron reaction, also the distribution of iron within the epithelium.


Dorsal tubule
Renal Corpusle.
1
Renal portal vein
Aorta.
Primary tubule
Post. vena cava.
Wolffian duct.
Neck.
Peritoneal canal.
Renal corpusle.
Nephrostome
4
5
3.
b.
2.
a3
b
a2
a1.
Ciliated neck
Mudrish

METHODS. In this study iron salts were used exclusively and were introduced into the animal by either of two methods, intraperitoneal or intravenous injection. A "balanced" mixture of sodium ferrocyanide and ammonium ferric citrate, made by adding 10 parts by volume of a 3 per cent solution of the former to 7 parts of a 4 per cent solution of the latter, was kept as a standard (4). The amount of iron salts to be injected into a given animal at one time was determined empirically. A portion of the standard solution was taken and diluted to such a degree with physiological salt solution that when 2 cc. of the diluted mixture were further diluted by an amount equal to the body volume of the animal it would give a decided Prussian blue reaction on the addition of hydrochloric acid. This was done to make the dosage uniform and also to ensure the microchemical detection of iron in the tissues. The amount to be injected by either route was fixed at 2 cc. to avoid producing intra-abdominal pressure in intraperitoneal injections and to prevent too great dilution of the blood when the intravenous route was used. At the close of the experiment the animals were anesthetized by immersion in $\frac{1}{4}$% per cent aqueous solution of chloretone and the entire blood supply to the kidney tied off. The kidneys were then dissected free and fixed in formalin acidified with 2 per cent hydrochloric acid. The Prussian blue reaction produced by this method is delicate and accurate as already demonstrated by Stieglitz (5) for the mammalian kidney.

Serial sections were prepared and stained with carmine for following the distribution of iron. For a study of histological details iron hematoxylin was used.

EXPERIMENTS. *Series I.* In the first series of experiments iron was introduced by single injections into the body cavity and the animals killed 2 to 24 hours later. Iron was present in the mesonephros but confined entirely to the primary and secondary tubules (those possessing peritoneal canals and nephrostomes), their collecting tubules and the Wolffian ducts. In preparations examined in toto under a binocular dissecting microscope the course of the primary and secondary tubules through the mesonephros can be readily followed due to the Prussian blue reaction. The only portions of the tubules that do not contain iron are the capsular spaces, and the proximal portions of necks lying between the renal corpuscles and the points at which the peritoneal canals join the renal tubules proper. When studied in sections iron is found as rather coarse particles in the lumina of all portions of the tubular system already mentioned, and intracellular iron can always be demonstrated in the proximal and occasionally in the distal convoluted portions. The greatest accumulation of iron within the cells is uniformly found in the proximal convoluted part, the iron in this region usually being concentrated near the free ends of the cells just beneath the "brush" border.

The iron particles in the lumen of the convoluted tubule also exhibited a tendency to be concentrated at the periphery of the lumen against the free border of the cells (fig. 3). In the animals killed 4 to 6 hours following the injection it was difficult to be positive as to the exact location of the iron, whetheri t was outside or just within the cells of the convoluted portion, but sections from animals killed 24 hours subsequent to the injection showed much of the iron well within the cells.

Series II. In intravenous injections, the iron was introduced through the abdominal vein. The vein was reached by an incision through the body wall slightly lateral to the mid-ventral line. Following the removal of the needle the vein was ligated on both sides of the perforation to prevent any oozing of iron and blood into the body cavity and the incision closed. Animals treated in this manner recovered from the anesthetic (chloretone) and remained active until killed from one to nine hours following the injection of the iron salts. Animals killed within less than an hour following injection did not show microscopically any iron in the kidney tissue and in most cases no distinctive blue reaction was obtained within less than two hours. After nine hours the entire mesonephros took on a deep blue color when fixed in acid formalin. The primary and secondary tubules appeared no denser than the dorsal tubules.

In microscopic preparations iron was found to be present in the lumina of all tubules, and intracellular iron was also seen in their convoluted portions (fig. 4). This result was in sharp contrast to that of the first series of experiments in which the distribution of iron was confined entirely to the primary and secondary tubules. For a time there was some uncertainty as to how the iron reached the lumina of the tubules. After a prolonged search through serial sections sufficient evidence was accumulated however to indicate that the iron was eliminated through the glomerulus. Iron particles in considerable numbers were found in the lumina of the ciliated necks in the region immediately beyond the point of transition from capsule to neck (fig. 2). The lumina of the capsules quite often appeared empty and the capsular space quite large due to the shrinkage following fixation. In many capsular spaces, however, fine granular masses usually adherent to the inner wall in the region of the apex of the glomerular tuft, were noted. In a few cases in which the granular precipitate was observed in the capsule lumen, relatively large quantities of iron were present in the glomerular vessels. Closer examination with oil immersion lenses revealed iron as very minute particles in both the endothelial cells of the glomerular vessels and the squamous cells of the visceral wall of the capsule as well as in the granular mass already described (fig. 2). There appeared to be a tendency for iron to be accumulated in some cells, more especially the endothelial cells, the accumulation being most pronounced along the basement membrane which separates the vascular endothelium from the glomerular epithelium.

Series III. Three animals were given repeated injections of iron intraperitoneally at intervals of 24 hours. The first two animals, a male and a female, received three successive injections and the third animal, a female, received five successive doses. All were killed twenty-four hours after receiving the last dose.

The kidneys of the first when dissected free and fixed according to routine showed the primary and secondary tubules very distinctly outlined and colored deep blue while the remaining portions of the kidneys were colored but a diffuse blue. The third animal which had received five repeated doses remained in good health, and the kidneys when fixed in acid formalin gave much the same color reaction as those of the first two animals, but the reaction throughout the entire kidney was more intense. Accordingly in this animal the difference in the intensity of the color reaction occurring in the primary and secondary tubules as contrasted with that in the more dorsal tubules was much less marked than it was in the two other animals.

Microscopic examination showed intracellular iron in all of the tubules and with the exception of the capsular spaces and tubular necks iron was present in all the portions of the tubules previously noted in series I and II. The amount of iron held in the proximal and distal convoluted portions of the primary and secondary tubules was far in excess of that found in similar locations in the dorsal tubules. It can also be stated that the amount of iron found within the cells of the convoluted portions of the primary and secondary tubules of animals receiving multiple injections intraperitoneally was many times more than that stored in the same cells of animals (series I) killed within a short time after receiving a single injection by the same route. In addition to this the iron found in the kidney cells in series III was distributed through the entire cell, showing also a tendency to accumulate at the bases of the cells rather than near the free margin (fig. 5), as described for series I and II.

Interstitial iron was also noted in the kidneys of the animals treated intraperitoneally with iron for rather long periods. It was especially noticeable in the regions of the convoluted portions of the primary and secondary tubules. Complete histological evidence as to the route followed by the interstitial iron is lacking. In some cells of both the proximal and distal convoluted portions, rather dense branching strands of iron granules can be traced through the cells from the free border to the base (fig. 6). The whole appearance rather suggests iron lying in either an intracellular or intercellular canalicular apparatus and it is usually in the vicinity of such cells that interstitial iron is found. Numerous leucocytes were also noted in the lumina of many primary and secondary tubules and in most instances their protoplasm was crowded with iron particles. The patches of interstitial iron accordingly may have been deposited by the activity of leucocytes.

The stomach, intestines and liver of the animals in series III gave a very definite iron reaction, but the body wall did not give any evidence of the absorption of iron from the body cavity. None of these organs were studied microscopically.

CONCLUSIONS AND DISCUSSION. The results of the three types of experiments outlined above, single intraperitoneal, single intravenous and repeated intraperitoneal injections of iron salts, lead us to certain conclusions regarding the course of iron through the mesonephros of Necturus. The results of series I show that iron which reaches the kidneys by way of the peritoneal canals is absorbed from the lumina of the tubules (primary and secondary) by the cells of both the proximal and distal convoluted portions. In this case there can be no question of secretion from the blood stream by way of the tubular epithelium since the iron salts were placed only in the body cavity, and the animal killed before sufficient time had elapsed for any absorption by the body wall or viscera.

In series II where the iron was introduced intravenously, it is found in all the renal tubules and is present intracellularly in the convoluted portions being found near the free border of the renal cells. Furthermore, since iron can be demonstrated in the endothelium of the glomerular vessels, in the squamous cells of the inner wall of the capsule, in the capsular space and in the ciliated neck of the tubule, it can be concluded that the iron salts passed into the renal tubules through the glomerulus and found their way into the convoluted epithelium in the same manner as they did when injected intraperitoneally, i.e., were absorbed from the lumina of the tubules.

In series III, repeated intraperitoneal injections, iron was found not only in the primary and secondary tubules but also in the dorsal tubules. The iron in the dorsal tubules must have been borne by way of the blood stream. From our observations it would appear that iron may pass into the blood by at least three ways: 1, transported from the lumina of the primary and secondary tubules by leucocytic activity; 2, absorbed from the lumina of the primary and secondary tubules by the epithelium of their convoluted portions and passed into the blood vessels, and 3, absorbed from the body cavity by the viscera, i.e., stomach, liver and intestine. There was no evidence of iron absorption by the peritoneum covering the body wall and mesonephros.

With repeated injections, the amount of iron stored in the cells of the dorsal tubules was much less than that in the cells of the primary and secondary tubules, but in all the tubules the iron particles tended to be concentrated at the bases of the cells, strongly favoring the interpretation of a movement of iron from the lumen of the tubules toward the blood stream. In this experiment it was difficult to demonstrate the passage of iron through the glomerulus. This was possibly due to the fact that

there was not sufficient iron passing to be detected microchemically. In the intravenous injections (series II) such large quantities of iron salts were being eliminated from the blood stream that they were more easily detected.

Our conclusions based on an amphibian mesonephros are almost diametrically opposed to those of Stieglitz (5) who concluded from his studies on the elimination of iron salts by the mammalian kidney that the iron is secreted by the tubular epithelium. Stieglitz's evidence is both negative and positive. He found no iron in the glomerular capsule or in the capsular space, but did note a definite movement of iron from the periphery of the cells of the convoluted tubules to the lumina of the tubules. We do not wish to give the impression, however, that in Necturus there is no evidence of any secretion by the tubular epithelium. On the contrary there is abundant evidence of secreting activity especially in the proximal convoluted portion, provided the distention of cells with granules is regarded as a sufficient criterion of such an activity. This phase of kidney activity has not received any attention in this present study.

Cushny believes that certain salt constituents of the urine as well as water are reabsorbed by the cells lining the kidney tubules. These substances have been spoken of as "threshold bodies" and it is believed that they only appear in the urine when the concentration in the blood stream rises above a certain level and their physiological threshold is supposed to be maintained to some extent by their reabsorption by the kidney tubules. Substances, such as sodium chloride, which are included in the group of "threshold bodies" appear to be essential to metabolic economy. In Necturus the iron salts appear to behave as "threshold bodies" and in addition to their reabsorption from the lumina of the tubules there also appears to be some storage within the kidney cells. This may be regarded as a factor of safety, the animal storing a reserve of such an essential element as iron when large amounts are being eliminated in the urine. When excessive amounts of iron are introduced by such a method as injection, the kidney cells hold excessive amounts of iron and thereby suffer injurious effects as is evidenced by the observations of Stieglitz (5) on mammals and by the presence of leucocytes and the occurrence of some desquamation in the tubules of Necturus.

It may be suggested, in such experiments as have been outlined above, that the large amount and unnatural form of the iron introduced into the body fluids established an unphysiological condition, and that such an excess may result in a diffusion of the iron, which would not occur if the material were present in a physiological state and amount. One must recognize the validity of such an objection but it is a criticism which seems inevitable in this type of experiment since sufficient iron must be introduced that it can be detected microchemically. On the other hand,

Stieglitz (5, p. 71) has suggested that iron introduced into the blood stream as salts may not remain as salts but be converted to colloidal iron or be loosely bound with serum proteins. If such a change does occur in the body fluids, diffusion cannot be urged as a criticism of the results obtained above.

SUMMARY

Two types of renal tubules are present in the mesonephros of Necturus: 1, primary and secondary tubules connected with the body cavity by peritoneal canals; and 2, other tubules, more dorsal in position, which lack peritoneal canals. Advantage is taken of this fact in an attempt to analyze renal activity.

Iron salts (intraperitoneal injection) which reach the lumina of the primary and secondary tubules directly from the body cavity are absorbed by the epithelium of their proximal and distal convoluted portions.

Iron salts which reach the mesonephros by the blood stream (intravenous injection) are eliminated through the glomeruli. Indirect evidence is also offered that the iron is reabsorbed by the epithelium of the convoluted portions.

With repeated intraperitoneal injections iron is found in the epithelium of the convoluted portions of all tubules, dorsal as well as primary and secondary. In these cells there is an accumulation of iron which is stored around the nuclei and at the bases of the cells.

With the exception of the capsule there is no evidence of any secretion of iron by the epithelium of any portion of the tubule.

BIBLIOGRAPHY

(1) Bieter and Hirschfelder: This Journal, 1924, lxviii, 326.
(2) Chase: Journ. Morphol., 1923, xxxvii, 457.
(3) Clark: Journ. Physiol., 1922, lv, 201.
(4) Collip: Univ. Toronto Studies, 1920, Physiological Series no. 35.
(5) Stieglitz: Amer. Journ. Anat., 1921, xxix, no. 1.

COMPARATIVE STUDY OF THE SUGAR CONCENTRATION IN ARTERIAL AND VENOUS BLOOD DURING INSULIN ACTION

CARL F. CORI AND GERTY T. CORI

From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.

Received for publication October 10, 1924

The first evidence of an extrahepatic action of insulin was given by Hepburn and Latchford (1), who found that insulin accelerated the rate at which glucose was removed by the excised mammalian heart from Locke's fluid. Mann and Magath (2) were able to show that the effect of insulin in producing hypoglycemia in dogs was not altered by the total removal of the liver. Cori, Cori and Goltz (3), (4) studied the action of insulin on the muscles of rabbits starved for 24 hours, by comparing the sugar concentration in the femoral artery and femoral vein. In 7 out of 13 experiments the difference in the sugar concentration between the inflowing and outflowing blood of the leg became distinctly larger after the subcutaneous administration of insulin. This was interpreted as evidence that the muscles withdraw more sugar than normal from the blood during insulin action. From the remaining 6 experiments, 3 showed a temporary increase of sugar intake, while 3 were negative. Cori, Pucher and Bowen (5) in a preliminary communication reported data on the influence of insulin on the difference in sugar concentration between arterial and venous blood of 7 diabetic and 3 normal individuals. It was found that 6 out of the 7 diabetics examined showed an increased intake of sugar by the muscles during insulin action. Frank, Nothmann and Wagner (6) from the Minkowski Clinic found later, in confirmation of our results, that by comparing the sugar concentration in the arterial and venous blood of rabbits a larger intake of sugar by the muscles can be demonstrated during insulin action. The difference between our experiments and those of Frank, Nothmann and Wagner was, that we administered the insulin subcutaneously while the latter authors injected it directly into the femoral artery. Frank, Nothmann and Wagner (7) obtained recently similar results on depancreatized dogs. The difference in sugar concentration between arterial and venous blood of the leg became much larger when insulin was injected into the femoral artery of the depancreatized animals. These authors interpreted their results as an indication that "insulin enables the muscles of the depancreatized animals

to withdraw sugar from the blood." Hepburn, Latchford, McCormick and Macleod (8) have been unable to demonstrate an increased disappearance of sugar from the blood into the muscles, as the result of insulin, by comparison of the sugar concentration in the arterial and venous blood of the muscles of anesthetized animals. The failure of Macleod and collaborators to obtain positive results seems to be due partly to the application of ether anesthesia and partly to the experimental technique itself, as will be discussed later.

It is the object of this paper to present new data that were obtained on rabbits receiving dextrose either alone or simultaneously with the insulin injection. The difference in sugar concentration between arterial and venous blood of the leg has again been used as an indication of the sugar intake by the muscles. The results were consistent and showed that the sugar intake by the muscles was stronger when dextrose plus insulin was given than when dextrose alone was fed. The data on the influence of insulin on the sugar concentration in the arterial and venous blood of the arm of diabetic patients have been withheld from detailed publication, since it was planned to carry out similar experiments on depancreatized animals, before presenting them. Since this has been done in the meantime by Frank, Nothmann and Wagner, it was decided to present the protocols on diabetic patients in this paper.

EXPERIMENTAL. Since nothing essential has been changed in the technique of obtaining blood from the femoral artery and femoral vein of unanesthetized rabbits, reference for the details of the description may be made to a previous publication (4). The femoral vessels in the present work were prepared under local anesthesia with 2 per cent novocain, generally on the day prior to the experiment. In two cases (tables 6 and 8) they were prepared 4 and $2\frac{1}{2}$ hours before the actual experiment was started. This could be done, since the operation did not cause an emotional hyperglycemia. Blood samples were taken in half-hour intervals and 0.3 and 0.4 cc. of blood were removed from each vessel. Since hemorrhages of any significance did not occur, the punctures could be repeated many times without causing any serious loss of blood to the animal. By using very fine needles and by handling the blood vessels very carefully and by closing the incision of the skin with a few stitches after the completion of each experiment, the rabbits could be used on two to three subsequent days. Since two assistants experienced in the handling of rabbits were present for each blood sampling, all operations could be performed with the greatest velocity. The time interval between the collection of blood from the femoral artery and from the femoral vein varied between 20 and 40 seconds, but was mostly 30 seconds. The rabbits were not tied on for more than 1 to 2 minutes and struggling of the animals could be prevented. Repeated blood sampling did not cause an emotional hyperglycemia.

The blood sugar was always determined in duplicate by the Hagedorn and Jensen (9) method. It was found by an experience of over 1000 determinations with this method that it is quite easy to obtain on duplicate determinations an agreement in the titration with 0.005 N thiosulfate of 0.02 cc. This corresponds to a mean error of ± 2 mgm. of blood sugar. It was made a rule that when duplicate determinations did not check within at least 0.02 cc. of the titration, the determination was repeated, so that the blood sugar values given in this paper have an error of maximally 2 per cent, but in many instances smaller than 2 per cent. The error for the difference between arterial and venous blood sugar would then be maximally 4 mgm., e.g., in the case that the error in the determination of the two types of blood lies in the opposite direction. Since the blood was analyzed as soon as it was drawn and since four determinations could easily be carried out by one worker in half an hour, no precautions against glycolysis were taken.

Experiments on rabbits. The experiments on rabbits (tables 1 to 11) were undertaken with the following object. In view of the effect of insulin on the sugar intake by the muscles of the starving and of the depancreatized animal, it seemed desirable to know whether animals receiving dextrose plus insulin would not show a stronger intake of sugar by the muscles than animals which received the same dose of dextrose but no insulin. As a standard dose 5 grams of Merck's anhydrous c.p. dextrose per kilogram were given to the rabbits by stomach tube. The insulin (iletin, Lilly) was from the same lot (U 20, lot no. 751369) and was injected intravenously. The rabbits were starved for 24 to 48 hours prior to the experiments.

Since sugar intake by the muscles is measured by the difference in sugar concentration between arterial and venous blood, it was necessary to ascertain whether repeated punctures themselves would not affect this difference. Consequently in 7 experiments (tables 3, 4, 9 and 10) several normal periods in half-hour intervals were made before glucose or glucose plus insulin was given. Since the individual variability of the test animal has to be taken into account, several comparative experiments, first with glucose alone and then with glucose plus insulin, were performed on the same rabbit. In one experiment this was reversed, so that first glucose plus insulin and then glucose alone was given to the same rabbit.

Discussion. It has been shown previously (4) that the normal difference between the blood sugar in the femoral artery and the femoral vein was 8 mgm. as an average of 20 observations, the maximum being 13 and the minimum 3. The present experiments comprise 40 observations on 12 rabbits all of which are not recorded in this paper, with an average difference of 6.3 mgm. (maximum 13, minimum 1). This gives a total average difference of 7 mgm. This figure is in agreement with that of

TABLE 1

Rabbit 2. Weight 2100 grams. On 8/22 femoral vessels prepared under local anesthesia. On 8/23 10 units insulin intravenously. On 8/25, 10.5 grams glucose by stomach tube. On 8/26, 10.5 grams glucose by stomach tube and 10 units insulin intravenously. Starved for 24 hours prior to each experiment.

Sugar per 100 cc. blood

MINUTES	A. INSULIN ALONE			B. GLUCOSE ALONE			C. INSULIN PLUS GLUCOSE		
	Femoral artery	Femoral vein	Difference (F.A.-F.V.)	Femoral artery	Femoral vein	Difference (F.A.-F.V.)	Femoral artery	Femoral vein	Difference (F.A.-F.V.)
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0	132	127	5	128	124	4	118	117	1
2	10 units insulin intravenously			10.5 grams glucose by stomach tube			10 units insulin intravenously, 10.5 grams glucose by stomach tube		
30	88	70	18	246	237	9	141	123	18
60	86	64	22	276	272	4	127	110	17
90	74	59	15	279	261	18	131	114	17
120	72	63	9	277	271	6	138	115	23
180				271	249	22	156	132	24

TABLE 2

Rabbit 4. Weight 2500 grams. On 8/26 femoral vessels prepared under local anesthesia. On 8/27, 12.5 grams glucose by stomach tube. On 8/29, 12.5 grams glucose by stomach tube and 10 units insulin intravenously. Starved for 24 hours prior to each experiment.

Sugar per 100 cc. blood

MINUTES	A. GLUCOSE ALONE			B. GLUCOSE PLUS INSULIN		
	Femoral artery	Femoral vein	Difference (F.A.-F.V)	Femoral artery	Femoral vein	Difference (F.A.-F.V.)
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0	114	103	11	132	127	5
3	12.5 grams glucose by stomach tube			12.5 grams glucose by stomach tube, 10 units insulin intravenously		
30	237	217	20	239	212	27
60	272	261	11	247	223	24
90	292	279	13	246	221	25
120	300	273	27	246	223	23
150	288	284	4			
180	266	255	11			
210	201	198	3			
240	151	149	2			

other workers, who determined the normal difference on unanesthetized animals and chose experimental conditions similar to ours. Thus Henriques and Ege (10) report 4 mgm. for dogs, Frank, Nothmann and Wagner (6) 4 to 6 mgm. for rabbits. However, Macleod and co-workers (8) found on dogs during ether narcosis a normal difference of 19 mgm. as an average of 14 experiments (maximum 57, minimum 0). It was stated previously (4, p. 370) that the absence of great variations in the normal

TABLE 3

Rabbit 9. Weight 2800 grams. On 9/3, femoral vessels prepared under local anesthesia. On 9/4, 14 grams glucose by stomach tube and 16 units insulin intravenously. On 9/6, 14 grams glucose by stomach tube. Starved for 48 hours prior to each experiment.

Sugar per 100 cc. blood

MINUTES	A. GLUCOSE PLUS INSULIN			B. GLUCOSE ALONE		
	Femoral artery	Femoral vein	Difference (F.A.-F.V.)	Femoral artery	Femoral vein	Difference (F.A.-F.V.)
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0	138	126	12	—	139	—
30	133	122	11			
60	132	126	6			
90	136	133	3			
120	137	135	2	151	143	8
150	137	130	7	151	149	2
153	14 grams glucose by stomach tube 16 units insulin intravenously			14 grams glucose by stomach tube		
180	97	74	23	235	215	20
210	104	79	25	271	243	28
240	105	84	21	275	261	14
270	97	77	20	261	235	26
300	90	83	7	255	249	6
330	93	77	16	248	236	12
360	99	79	20	232	223	9

difference enabled us to study whether the muscles participate in any way in bringing about the fall of blood sugar during insulin action. The wide variations in the normal difference between arterial and venous blood sugar which are obtained with the technique of Macleod and Pearce (11) and Macleod and Fulk (12) are not favorable enough to study the effect of insulin on the sugar intake by the muscles and the results are bound to be inconclusive. It is therefore not surprising that Macleod obtained entirely negative results in his insulin experiments. Even though our technique gives quite constant values for the normal difference, whether a series of rabbits or the same rabbit on subsequent days is used, it was thought advisable to investigate what fluctuations in the

TABLE 4

Rabbit 11. Weight 2700 grams. On 9/8, femoral vessels prepared under local anesthesia. On 9/9, 13.5 grams glucose by stomach tube. On 9/10, 13.5 grams glucose by stomach tube and 18 units insulin intravenously. Starved for 48 hours prior to each experiment. On both experimental days the temperature of the laboratory was very cold due to a sudden change in the weather.

Sugar per 100 cc. blood

MINUTES	A. GLUCOSE ALONE			B. GLUCOSE PLUS INSULIN		
	Femoral artery	Femoral vein	Difference (F.A.-F.V.)	Femoral artery	Femoral vein	Difference (F.A.-F.V.)
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
0	134	127	7	150	144	6
30	157	154	3	160	154	6
60	150	142	8	162	152	10
90	154	153	1	155	153	2
92	13.5 grams glucose by stomach tube			13.5 grams glucose by stomach tube, 18 units insulin intravenously		
120	247	215	32	97	66	31
150	271	253	18	101	76	25
180	290	271	19	106	81	25
210	291	272	19	109	74	35
240	252	233	19	114	88	26
270	233	220	13	114	92	22
300	219	204	15	110	98	12
330	185	180	5	117	98	19

TABLE 5

Rabbit 1. Weight 1800 grams. On 8/21, femoral vessels prepared under local anesthesia. On 8/23, 9 grams glucose by stomach tube. Starved for 48 hours prior to the experiment.

Sugar per 100 cc. blood

MINUTES	FEMORAL ARTERY	FEMORAL VEIN	DIFFERENCE (F.A.—F.V.)
	mgm.	*mgm.*	*mgm.*
0	127	119	8
3	9 grams glucose by stomach tube		
30	217	197	20
60	206	202	4
90	207	192	15
150	190	170	20
210	147	137	10

difference between arterial and venous blood sugar may occur when repeated punctures in short time intervals are made. Since it seems that none of the workers who used a comparison of arterial and venous blood

TABLE 6

Rabbit 3. Weight 2050 grams. On 8/25, at 10 a.m., femoral vessels prepared under local anesthesia. At 2 p.m., 10 grams glucose by stomach tube. Starved for 24 hours prior to the experiment.

Sugar per 100 cc. blood

MINUTES	FEMORAL ARTERY	FEMORAL VEIN	DIFFERENCE (F.A. – F.V.)
	mgm.	*mgm.*	*mgm.*
0	135	132	3
5	10 grams glucose by stomach tube		
30	221	200	21
60	251	244	7
90	249	229	20
120	229	216	13
150	209	197	12
180	201	200	1
240	184	183	1

TABLE 7

Rabbit 6. Weight 1500 grams. On 8/29, femoral vessels prepared under local anesthesia. On 8/30, 7.5 grams glucose by stomach tube and 8 units insulin intravenously. Starved for 48 hours prior to the experiment.

Sugar per 100 cc. blood

MINUTES	FEMORAL ARTERY	FEMORAL VEIN	DIFFERENCE (F.A. – F.V.)
	mgm.	*mgm.*	*mgm.*
0	145	132	13
5	7.5 grams glucose by stomach tube 8 units insulin intravenously		
30	170	143	27
60	160	139	21
90	155	130	25
120	154	125	29
150	157	138	19
180	156	142	14
210	148	139	9
240	151	143	8

took a sufficient number of samples previous to an experiment, such data have been included in this paper. Tables 3, 4, 9 and 10 show that repeated blood sampling does not produce a greater variation in the difference

TABLE 8

Rabbit 7. Weight 2500 grams. On 9/2, at 9 a.m., femoral vessels prepared under local anesthesia. At 11:30 a.m., 12.5 grams glucose by stomach tube and 14 units insulin intravenously. Starved for 48 hours prior to the experiment.

Sugar per 100 cc. blood

MINUTES	FEMORAL ARTERY	FEMORAL VEIN	DIFFERENCE (F.A. – F.V.)
	mgm.	*mgm.*	*mgm.*
0	123	118	5
2	12.5 grams glucose by stomach tube 14 units insulin intravenously		
30	91	64	27
60	84	68	16
90	92	70	22
120	94	73	21
150	104	84	20
180	106	88	18
210	125	112	13

TABLE 9

Rabbit 8. Weight 1500 grams. On 9/2, femoral vessels prepared under local anesthesia. On 9/3, 7.5 grams glucose by stomach tube and 8 units insulin intravenously. Starved for 48 hours prior to the experiment

Sugar per 100 cc. blood

MINUTES	FEMORAL ARTERY	FEMORAL VEIN	DIFFERENCE (F.A. – F.V.)
	mgm.	*mgm.*	*mgm.*
0	111	101	10
30	118	116	2
60	127	119	8
90	130	126	4
120	125	121	4
150	129	121	8
155	7.5 grams glucose by stomach tube 8 units insulin intravenously		
180	127	100	27
210	120	91	29
240	99	80	19
270	97	73	24
300	99	72	27
330	93	73	20
360	104	84	20

between arterial and venous blood sugar than is normally occurring nor does it cause an emotional hyperglycemia.

Since in recent times an increasing number of investigators have used a comparison of the in- and outflowing blood of an organ as a fruit-

TABLE 10

Rabbit 12. Weight 2350 grams. On 9/15, femoral vessels prepared under local anesthesia. On 9/16, 11.7 grams glucose by stomach tube. On 9/17, 11.7 grams glucose by stomach tube and 20 units insulin intravenously. Starved for 48 hours. This experiment differs from the preceding ones in that the blood samples were all taken within the first 30 minutes after the glucose ingestion

Sugar per 100 cc. blood

MINUTES	FEMORAL ARTERY	FEMORAL VEIN	DIFFERENCE (F.A.-F.V.)	MINUTES	FEMORAL ARTERY	FEMORAL VEIN	DIFFERENCE (F.A.-F.V.)
	mgm.	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*
0	134	130	4	0	148	140	8
29	141	135	6	28	158	148	10
30	11.7 grams glucose by stomach tube			30	11.7 grams glucose by stomach tube; 20 units intravenously		
39	181	161	20	38	208	191	17
47	202	181	21	47	215	195	20
56	218	211	7	55	216	181	35
61	244	223	21	61	191	168	23

TABLE 11

Summary of the data of tables 1 to 9. The difference between arterial and venous blood sugar that were observed 30, 90 . . . . 240 minutes after giving either glucose alone or glucose plus insulin are recorded and the average differences for each observation time are calculated

MINUTES	A. GLUCOSE ALONE		B. GLUCOSE PLUS INSULIN	
	Difference (F. A.-F. V.)	Average difference (F.A.-F.V.)	Difference (F. A.-F. V.)	Average difference (F.A.-F.V.)
	mgm.	*mgm.*	*mgm.*	*mgm.*
30	9; 20; 20; 32; 20; 21	20.3	18; 27; 23; 31; 27; 27; 27	25.7
60	4; 11; 28; 18; 4; 7	12.0	17; 24; 25; 25; 21; 16; 29	22.4
90	18; 13; 14; 19; 15; 20	16.5	17; 25; 21; 25; 25; 22; 19	22.0
120	6; 27; 26; 19; 13	18.2	23; 23; 20; 35; 29; 21; 24	25.0
150	4; 6; 19; 20; 12	12.2	7; 26; 19; 20; 27	19.8
180	22; 11; 12; 13; 1	11.8	24; 16; 22; 14; 18; 20	19.0
210	3; 9; 15; 10	9.2	20; 12; 9; 13; 20	14.8
240	2; 5 1	2.7	19; 8	13.5

ful method for the investigation of biological problems, a few remarks on the technique of such experiments would seem in place. The first requirement in work on carbohydrate metabolism would be to use unnarcotized animals. Since the circulation through the organs should be

disturbed as little as possible, blood should be obtained by puncture with fine needles rather than by inserting cannulae in the main vessels and the arterial and venous blood should be drawn as simultaneously as possible. In the technique of Macleod (8) long glass cannulae were inserted in the central ends of the femoral artery and vein on one side, being pushed up, so that the open ends lay in the iliac vessels close to the abdominal aorta and vena cava. Ligatures were then tied round the cannulae near their open ends. However, the venous blood thus collected is a mixture of venous blood from the opposite leg and from the organs of the pelvis. Macleod stated (8, p. 557) that many of his experiments were inconclusive because of unsurmountable difficulties in securing samples of blood from all the vessels at approximately the same time. This was particularly the case with the collection of blood from the vena cava, in which vessel a cannula was introduced by way of the central end of the renal vein, being pushed up, so that the open end lay opposite to the hepatic veins (for collection of blood from the liver). Bang (13) has already criticised the use of similar methods, in which an introduction of a catheter into the internal veins not only involved a laparotomy and manipulations in the abdominal cavity, but caused a serious circulatory disturbance in the animal. In Macleod's technique the vein of one kidney is entirely obstructed, which undoubtedly seriously injures this organ. But by merely placing the open end of a cannula in the vena cava opposite to the liver veins it seems hardly possible to obtain blood from the liver without admixture of blood from the vena cava. In a method described previously (4) the liver vein of unnarcotized rabbits was punctured directly, avoiding an admixture of blood from the vena cava. It was found in a previous, unpublished experiment, that the blood sugar of the liver vein was 0.157 per cent, that of the femoral artery 0.133 per cent, or a difference of 24 mgm. in favor of the former, while blood obtained simultaneously from the vena cava opposite to the liver veins contained only 0.141 per cent sugar. Macleod called attention to the fact that Lépine and Boulud (14) found exactly the opposite relationship between the sugar in blood samples taken simultaneously from the carotid artery and right ventricle, than we found between liver vein and femoral artery, viz., an average of 0.023 per cent more in the former. In studying this reference it appeared that the results of Lépine and Boulud were constant only when dogs were used in which a hyperglycemia was produced by partial asphyxia, chloroform or alcohol, or a hypoglycemia by phlorhizin, while irregular data were obtained on the normal animal. Since it was not easy to conceive how more sugar could appear in the blood after its passage through the lungs, Lépine resorted to the theory that part of the "sucre virtuel" was liberated in the capillaries of the lungs, thus augmenting the sugar concentration in the carotid artery over that in the right ventricle.

Apart from other considerations a connection between our data and those of the French authors is not apparent, since the blood was obtained from different sources, viz., from the hepatic vein and femoral artery in our experiments and from the right ventricle and carotid artery in those of Lépine and Boulud. An interesting technique for the collection of blood from the liver vein and from the portal vein has been recently developed by London (15). In a preliminary operation a metal tube of small diameter is inserted in such a way that one open end is held in close contact with one liver vein, while the other opening is inserted into the incision of the skin. A similar tube serves for the portal vein. The animals seem to survive this operation indefinitely and are perfectly normal. Blood can be obtained, whenever it is desired, by direct puncture of the vessel through the metal tube and observations can be extended over long periods of time, which is a great advantage. If the technical difficulties of the preliminary operation are not too great, this would be indeed the ideal method.

The experiments with rabbits are summarized in table 11, where the average differences between arterial and venous blood sugar for the series with insulin plus glucose and for the series with glucose alone are calculated. It will be seen from this table that the average differences of the former series are for all observation times greater than those of the latter series. This would mean that more sugar disappears from the blood into the muscles in the former instance than in the latter, or that insulin causes an increased intake of sugar by the muscles. In analyzing this table more in detail, it will be noted that there is a break in the curve of the sugar intake by the muscles at 60 minutes, the difference between arterial and venous blood being only 12.0 mgm., while this break almost disappears under the influence of insulin. When the individual values for the same observation time are compared with each other a far greater variability is seen in the series with sugar alone than in the series with glucose plus insulin. An examination of tables 1 to 9 shows that the curve for the alimentary hyperglycemia of rabbits receiving 5 grams of glucose per kilogram shows a steep rise in the first 30 minutes after the glucose injection. This is followed by a distinct plateau lasting for about 3 hours and by a less steep decline after 3 to 3½ hours. On observing the effect of insulin on this curve it will be noted that with one exception (table 2) enough insulin had been given to prevent the steep rise in blood sugar within the first 30 minutes as well as the hyperglycemia in the subsequent periods. Since the greatest alteration in the curve of the alimentary hyperglycemia was produced by insulin within the first half-hour, it seemed of interest to study the changes in the difference between arterial and venous blood sugar within this period, by taking blood samples at very short time intervals. One experiment of this type has been

performed (table 10) in which, owing to the rapid changes in blood sugar within this period, special precautions were taken to withdraw arterial and venous blood at the same rate. This was accomplished by dividing the work of the blood collection between two persons, one drawing the arterial blood and the other the venous blood as simultaneously as possible. Not enough insulin was given to this animal to entirely prevent the rise in blood sugar. It was found that within 8 to 9 minutes after the glucose ingestion an increase in the difference between arterial and venous blood sugar occurred. The effect of insulin, however, in producing an increased rate of disappearance of sugar from the blood into the muscles was not noticeable until 26 minutes after the glucose ingestion. In one experiment (table 1) a comparison of arterial and venous blood sugar was made on a starving rabbit 30 minutes after the injection of insulin, the difference between arterial and venous blood sugar rose to 18 mgm. and remained above normal for 90 minutes.

Taking the results as a whole, they are in agreement with our former contention that insulin leads to an increased sugar intake by the muscles. In this connection, the experience of Marriott (16) that malnourished infants gain rapidly in weight when they receive for several days glucose and insulin intravenously, is of a certain interest. There is no doubt that the utilization of glucose must be preceded by its penetration into the tissue cells and it is probable that the increased disappearance of sugar from the blood into the muscles is the consequence of an increased rate of metabolization of glucose in the muscles during insulin action. It is, however, not known how the sugar that disappears into the muscles is disposed of. In the absence of appropriate quantitative experiments, which should account for the oxidative and synthetic disposal of the sugar simultaneously, it can only be stated that probably part of the sugar is oxidized and part of the sugar is utilized for synthetic processes.

The data of the literature, with the exception of those of Macleod, all point in the direction that insulin leads to an increased disappearance of sugar from the blood into the muscles. On former occasions, however, Macleod (17) has expressed himself in favor of this conception. He stated (p. 49): "Although for various reasons, it would be of considerable interest to be able to demonstrate that insulin affects the rate of disappearance of glucose from fluid perfused through skeletal muscle, as can be done in the case of fluids perfused through the heart, the demonstration is not essential as proof that it has this effect." Frank, Nothmann and Wagner (6) suggested that some of our former experiments on starving animals might have been negative because the insulin was injected subcutaneously, in which case it would be distributed immediately in the whole body and would not be concentrated enough in the local vascular area of the leg. This consideration led these authors to the intra-arterial

application of insulin and they claimed that by doing so they have obtained more consistent results. Macleod (8) having obtained entirely negative results, thought that the method of comparison of the blood sugar in different vessels is of no value and he stated that our conclusions, that insulin leads to a diminished output of sugar by the liver and to an increased intake of sugar by the muscles, were not justified by our experimental results. In the first group of our former experiments a comparison of the sugar concentration in the hepatic vein and in the jugular vein was made and the results obtained suggested that a retention of sugar by the muscles plays an important part during insulin action. In order to test this possibility a comparison of the sugar concentration in the femoral artery and femoral vein was made in the second group of experiments. It was found that insulin may increase the difference between arterial and venous blood sugar and it followed from this that the sugar output by the liver could not be measured by a comparison of the sugar concentration in the liver vein and a peripheral vein. The sugar output by the liver can therefore be studied only in the third group of experiments, where a comparison of the sugar concentration in the hepatic vein, the femoral artery and the femoral vein was made simultaneously. Macleod analyzed in detail the data of the first and second group of experiments, but did not more than allude to the third and most important group of experiments, from which most of the conclusions were drawn. In the first group 5 representative experiments out of 9 were recorded. Three protocols were given to illustrate that the difference in sugar concentration between hepatic vein and jugular vein may diminish during insulin action, while two protocols were recorded to show that the difference may remain the same or may even increase after the administration of insulin. From the former 3 protocols one should be ruled out, according to Macleod, because less insulin was injected than in the other experiments, in which another lot of insulin was employed. But since the dose given (1 unit intravenously into a rabbit weighing 1600 grams) caused a fall in blood sugar to 54 mgm. one hour after the injection, it is not clear why this experiment should not be used. In the other protocols (nos. 3 and 4) where the difference in the sugar concentration between liver vein and neck vein became decidedly smaller after the injection of insulin, this could have been due, according to Macleod, to a lessened retention of glucose in the vascular area drained by the neck veins and would therefore not be a proof that insulin leads to a diminished output of sugar by the liver into the blood stream. Since it was stated on page 365 that "if a larger amount of sugar is taken up from the blood by the muscles during insulin action, a comparison of the blood sugar concentration in the liver vein and neck vein does not present a true picture of the actual sugar output by the liver," it was evident that no final conclusions on the sugar

output by the liver were drawn from these experiments. In fact, the conclusions were drawn from the experiments of the third group, where the comparison of the sugar concentration in the hepatic vein and femoral artery showed that the difference in sugar concentration between these two types of blood was decidedly diminished in all six experiments performed. In the last experiment the decrease of the difference did not take place immediately after the insulin injection, but was marked in the third and fourth hour, when the blood sugar stood at 75 and 65 mgm. respectively. Macleod by not quoting these experiments made it appear as if the only experiments in which the sugar output by the liver was studied, were those criticised by him. In the second group 7 experiments were recorded, which were interpreted by Macleod as follows, namely, that in three experiments the maximum difference found in normal animals was exceeded after insulin, while in the rest of the experiments the differences were within the normal limits or below in one case. There are, however, 6 more experiments in the third group in which a comparison of the sugar concentration between leg artery and leg vein were made, which were not mentioned by Macleod. In 4 of these experiments an increase in the difference between arterial and venous blood sugar after the injection of insulin was noted, while one showed a temporary increase and one was below the normal limit. Macleod finally raised the objection that the blood pressure was not recorded in our experiments. Since it was decided to use unnarcotized animals this was not possible, nor did it seem necessary since Edwards and Page (18) have shown that the mean blood pressure shows only a moderate decline during the first 2 hours following large doses of insulin. In four of Macleod's protocols, blood pressures as low as 30 mm. Hg are recorded shortly after the injection of insulin when the blood sugars in the vein stood at 55, 140, 138 and 83 mgm. respectively, but since the blood sugar was not very low, or indeed normal in two cases and since the animals were in ether narcosis and were subjected to a laparotomy, it does not follow that the fall in blood pressure was due to insulin.

The data presented in this paper show that when sugar plus insulin was given, or in other words when a certain excess of sugar was present, an increase in the sugar intake by the muscles could be demonstrated in all experiments with great regularity. Since the sugar intake by the muscles is undoubtedly dependent on the amount of sugar available it seems likely that our former results obtained on rabbits starved for 24 hours were not consistent, because variable amounts of sugar were available to each rabbit. It is well known that rabbits from which food has been withheld for 24 hours still contain a considerable amount of foodstuffs in the stomach and in the intestines and this amount might have varied for each rabbit. This explanation seems more probable

than the one given by Frank, Nothmann and Wagner, that the irregular results were due to the subcutaneous administration of insulin.

Experiments on normal men and on diabetic patients. For the collection of arterial and venous blood from the patients a technique similar to that of Lundsgaard and Möller (19) and of Foster (20) was used. The patients, who were at rest before and during the experiment, were induced to hold their finger tips for 5 minutes in warm water so as to produce a mild degree of hyperemia. One finger tip was then cleaned with alcohol and a fairly deep prick was made with a spring lancet. The first drop of blood was discarded and if the blood did not drop out freely, this was facilitated by a gentle massage of the finger, but squeezing was avoided in all cases. While the collection of blood from the finger was carried out by an assistant, a fine needle was introduced into the cubital vein of the same arm and the venous blood collected without stasis at about the same rate as the blood from the fingertip. Lundsgaard and Möller have shown that arterial blood (from the radial artery) and cutaneous blood (from the finger tip) have the same oxygen content and they assumed that this identity of arterial and cutaneous blood could be extended to the content of sugar, salt, uric acid, etc., and also to the reaction of the blood. Hagedorn (21) found that cutaneous blood differs from venous blood in its sugar concentration and used the hypothesis that the cutaneous blood is similar to arterial blood in that respect. Foster (20) found in two dogs, after a sugar meal, a difference between arterial blood and cutaneous blood from the foot of 2 and 5 mgm. respectively, while the sugar concentration in the vein was 34 and 24 mgm. lower than in the artery. From these data of the literature it seems justified to refer to the cutaneous blood from the finger as arterial blood.

A comparison of arterial and venous blood sugar concentration was made on 16 normal persons. The subjects were mostly laboratory workers, who volunteered. Blood was taken 3 to 4 hours after a light breakfast. The average sugar content of the arterial blood was 0.1035 per cent and of the venous blood 0.098 per cent. This gives an average difference of 5.5 mgm., the maximum being 9 and the minimum 2 mgm. In three normal persons the changes in the difference following the injection of insulin were studied and in one case 100 grams glucose were ingested.

It will be seen from table 12 that the normal difference was exceeded three times in blood samples taken 30 to 60 minutes after the insulin injection. This would mean that an increased intake of sugar by the muscles can also be demonstrated in normal men during insulin action. The data of Foster (20) that the ingestion of 100 grams glucose by normal men leads to an increase in the difference between arterial and venous blood sugar are confirmed, but it seems possible that the very large differ-

ences, up to 80 mgm., that were observed by Foster were due to the fact that the venous blood was collected 5 minutes later than the arterial blood from the finger. This would tend to increase the difference, especially at the time of the rapid return of the blood sugar to normal.

The difference between arterial and venous blood sugar was studied on 24 diabetic patients with a total number of 67 observations. The blood was collected on the days that were designed in the hospital routine

TABLE 12

The influence of insulin and of sugar ingestion on the difference between arterial and venous blood sugar concentration of normal men

EXPERIMENT NUMBER	DATE	Sugar per 100 cc. blood: ARTERIAL BLOOD	VENOUS BLOOD	DIFFERENCE (A.Bl.–V.Bl.)	REMARKS
		mgm.	*mgm.*	*mgm.*	
1	11/ 2/23	95	92	3	Before insulin
		91	76	15	60 minutes after insulin (7 units subcutaneously)
		83	78	5	120 minutes after insulin
2	11/ 2/23	101	96	5	Before insulin
		98	82	16	60 minutes after insulin (8 units subcutaneously)
		111	105	6	120 minutes after insulin
3	10/31/23	122	117	5	Before insulin
		102	88	14	30 minutes after insulin (10 units subcutaneously)
		96	80	16	60 minutes after insulin
		97	87	10	120 minutes after insulin
4	8/22/24	116	109	7	Before glucose
		165	135	30	30 minutes after the ingestion of 100 grams glucose
		203	175	28	60 minutes after glucose ingestion
		167	146	21	90 minutes after glucose ingestion
		137	119	18	120 minutes after glucose ingestion

for blood sugar tests, the patients having received their insulin dose and their breakfast 2 to 3 hours previously. The difference varied from 0 to 28 mgm., but was mostly higher than 5.5 mgm., the average found for normal persons. Owing to the difficulty of obtaining untreated diabetic patients of a more severe type only a limited number of observations could be made on such patients. In three untreated cases the difference was very small, ranging from 0 to 3 mgm. Experiments were carried out on 8 diabetic patients, in which the difference between

TABLE 13

The influence of insulin and of sugar ingestion on the difference between arterial and venous blood sugar concentration of diabetic patients

Sugar per 100 cc. blood

EXPERIMENT NUMBER	DATE	ARTERIAL BLOOD	VENOUS BLOOD	DIFFERENCE A.Bl.–V.Bl.	REMARKS
		mgm.	*mgm.*	*mgm.*	
1	10/30/23	330	330	0	Before insulin. (Moderately severe, untreated diabetes)
		284	252	32	60 minutes after insulin (20 units subcutaneously)
		164	129	35	120 minutes after insulin
		105	93	12	150 minutes after insulin
	11/ 7/23	214	211	3	Before insulin. (Merely dietary treatment since last experiment)
		167	151	16	60 minutes after insulin (16 units subcutaneously)
		115	102	13	120 minutes after insulin
		94	93	1	150 minutes after insulin
2	10/31/23	152	144	8	Before insulin. (Mild diabetes. Treated case)
		125	108	17	60 minutes after insulin (10 units subcutaneously)
		110	93	17	120 minutes after insulin
3	10/26/23	163	157	6	Before insulin. (Moderately severe, treated case)
		118	103	15	60 minutes after insulin (13 units subcutaneously)
		78	63	15	120 minutes after insulin. Symptoms of hypoglycemia
		73	73	0	150 minutes after insulin
	11/19/23	138	132	6	Before glucose
		372	338	34	60 minutes after ingestion of 50 grams glucose
		314	298	16	120 minutes after glucose
		256	254	2	150 minutes after glucose
4	10/26/23	275	265	10	Before insulin. (Severe, treated case. Died later in diabetic coma)
		236	227	9	60 minutes after insulin (20 units subcutaneously)
		183	179	4	120 minutes after insulin
		136	136	0	150 minutes after insulin
	11/ 9/23	308	295	13	Before glucose
		408	376	32	60 minutes after ingestion of 30 grams glucose
		348	339	9	120 minutes after glucose
		328	316	12	150 minutes after glucose
	12/11/23	280	284	+4	Before insulin
		269	271	+2	60 minutes after insulin (16 units subcutaneously)
		231	225	6	120 minutes after insulin
		192	187	5	150 minutes after insulin

TABLE 13—*Concluded*

EXPERIMENT NUMBER	DATE	ARTERIAL BLOOD	VENOUS BLOOD	DIFFERENCE A.Bl.-V.Bl.	REMARKS
		mgm.	*mgm.*	*mgm.*	
5	11/ 1/23	142	138	4	Before insulin. (Mild, treated case. Arteriosclerosis)
		111	99	12	60 minutes after insulin (10 units subcutaneously)
		105	94	11	120 minutes after insulin
6	12/ 3/23	152	147	5	Before insulin. (Mild, treated case)
		123	106	17	60 minutes after insulin (10 units subcutaneously)
		103	93	10	120 minutes after insulin
7	11/ 4/23	295	294	1	Before insulin. (Moderately severe, untreated case)
		239	218	21	60 minutes after insulin (20 units subcutaneously)
8	9/ 8/24	290	288	2	Before insulin. (Moderately severe, untreated case)
		231	221	10	85 minutes after insulin (22 units subcutaneously)
		158	142	16	155 minutes after insulin
		130	120	10	205 minutes after insulin

arterial and venous blood sugar was followed after the injection of insulin and as a comparison glucose was given in two cases, the breakfast and the insulin dose in the morning having been omitted previously.

Wertheimer (22) determined the difference between arterial and venous blood sugar in diabetic patients and depancreatized dogs, using the Bertrand method on 20 cc. blood. The arterial blood from the patients was obtained by puncture of the radial artery. On normal persons a difference of 11.6 mgm. was found as an average of 9 observations, while in 6 untreated diabetic patients the difference varied from 0 to 3 mgm. Five dogs showed an average difference of 12.8 mgm., while the same dogs after pancreatectomy had a difference ranging from 0 to 4 mgm. The data of Macleod and collaborators (8) showed a variation for the difference of depancreatized dogs during ether narcosis from 3 to 85 mgm. Since even insulin doses as high as 470 units did not reduce the blood sugar of these etherized animals, the observations on the changes in the difference following insulin were inconclusive. Frank, Nothmann and Wagner (7) found in 5 diabetic dogs no difference in two cases and a higher sugar concentration in the vein than in the artery in three cases. After an insulin injection into the femoral artery the difference between arterial and venous blood sugar was increased on the side of the injection and reached values as high as 42 mgm. The increase lasted for 4 to 5 hours. The experiments on diabetic patients in table 13 show likewise that in-

sulin leads to an increase in the difference between arterial and venous blood sugar concentration. This would mean that insulin enables the muscles of diabetic patients to withdraw more sugar from the blood, but it is realized that experiments on diabetic patients are less favorable than on depancreatized animals, where the observations can be made on completely diabetic organisms. In one case (no. 4) a negative result was obtained and this was duplicated in a second experiment. To this and another diabetic patient of a more severe type (no. 3) glucose was given, with the effect that the difference between arterial and venous blood sugar increased, indicating that the muscles of these patients were still able to withdraw sugar from the blood in increased amounts when the blood sugar concentration was sufficiently raised.

SUMMARY AND CONCLUSIONS

1. The difference in the sugar concentration between the femoral artery and femoral vein of rabbits starved previously for 24 to 48 hours was found to be 7 mgm. as an average of 60 observations on 25 rabbits (maximum 13, minimum 1).

2. After an ingestion of 5 grams glucose per kilogram the difference between arterial and venous blood sugar was increased during the period of hyperglycemia, indicating that more sugar than normal was withdrawn by the muscles from the blood.

3. After glucose plus insulin (5 grams glucose per kilogram and an insulin dose sufficiently large to prevent a rise in blood sugar) the increase in the difference between arterial and venous blood sugar was greater and lasted longer than under the conditions mentioned under 2. This was interpreted as further evidence that insulin leads to an increased disappearance of sugar from the blood into the muscles.

4. The difference in the sugar concentration between cutaneous blood from the finger tip (arterial blood) and blood from the cubital vein in 16 normal persons averaged 5 mgm. (maximum 9, minimum 2). In three normal persons this average value was exceeded three times 30 and 60 minutes after the insulin injection.

5. The difference between arterial and venous blood sugar was higher for diabetic patients, who received insulin treatment than for untreated cases. Thus in 24 diabetic patients during treatment 2 to 3 hours after breakfast and an insulin injection, the differences ranged from 0 to 28 mgm., while in three untreated, moderately severe diabetic patients the differences ranged only from 0 to 3 mgm. The changes in the difference between arterial and venous blood sugar following insulin were studied in detail on 8 diabetic patients, 7 of whom showed an increase in the difference after the insulin injection. Glucose was given in doses of 30 to 50 grams to two diabetic patients of a more severe type and an increase in the difference between arterial venous blood sugar was noted.

6. It is concluded that insulin increases the rate at which sugar disappears from the blood into the muscles, in the partially starved rabbit, in the sugar fed rabbit, in normal men and in diabetic patients.

We wish to thank Doctor Bowen for permitting us to use diabetic patients from the Buffalo General Hospital for this work, and Doctor Pucher for the collection of many blood samples. We also wish to express our appreciation of the assistance rendered in the animal experiments by Miss H. Goltz and Miss H. DeNiord. The insulin used in this work was supplied, free of charge, by Ely Lilly & Company, Indianapolis.

While this paper was in press there appeared a communication by Burn and Dale (*Journ. Physiol.*, 1924, lix, 164), who used decapitated, eviscerated and in some instances skinned preparations of cats. In one series glucose alone and in a second series glucose plus insulin was infused intravenously. In the latter series 2 to 4 times more glucose disappeared from the blood than in the former. Burn and Dale concluded from their experiments that insulin leads to an increased disappearance of sugar from the blood into the muscles.

BIBLIOGRAPHY

(1) Hepburn and Latchford: This Journal, 1922, lxii, 177.
(2) Mann and Magath: Ibid., 1923, lxv, 403.
(3) Cori, Cori and Goltz: Proc. Soc. Exper. Biol. and Med., 1923, xxi, 121.
(4) Cori, Cori and Goltz: Journ. Pharm. Exper. Therap., 1923, xxii, 355.
(5) Cori, Pucher and Bowen: Proc. Soc. Exper. Biol. and Med., 1923, xxi, 122.
(6) Frank, Nothmann and Wagner: Klin. Wochenschr., 1924, iii, 581.
(7) Frank, Nothmann and Wagner: Klin. Wochenschr., 1924, iii, 1404.
(8) Hepburn, Latchford, McCormick and Macleod: This Journal, 1924, lxix, 555.
(9) Hagedorn and Jensen: Biochem. Zeitschr., 1923, cxxxv, 46.
(10) Henriques and Ege: Ibid., 1921, cxix, 121.
(11) Macleod and Pearce: This Journal, 1916, xxxviii, 415, 425.
(12) Macleod and Fulk: Ibid., 1916, xlii, 193.
(13) Bang: Der Blutzucker, Wiesbaden, 1913.
(14) Lépine: Le Diabète Sucré, Paris, 1909, 66–69.
(15) London: Pflüger's Arch., 1923, cci, 360.
(16) Marriott: Journ. Amer. Med. Assoc., 1924, lxxxiii, 600.
(17) Macleod: Physiol. Reviews, 1924, iv, 21.
(18) Edwards and Page: This Journal, 1924, lxix, 177.
(19) Lundsgaard and Möller: Journ. Exper. Med., 1922, xxxvi, 559.
(20) Foster: Journ. Biol. Chem., 1923, lv, 291, 303.
(21) Hagedorn: Ugesk. Laeger, 1920, lxxxii, 796.
(22) Wertheimer: Med. Klinik, 1924, xx, 632.

THE INSULIN CONTENT OF THE PANCREAS AND OTHER TISSUES IN ANIMALS POISONED WITH PHLORHIZIN

GERTY T. CORI

From the State Institute for the Study of Malignant Disease, Buffalo, N.Y.

Received for publication October 10, 1924

It seems well established that an animal in the state of complete phlorhizin poisoning suffers from two incapacities. One is the defect of the kidney to resorb the sugar that is filtered from the blood. This defect leads to glycosuria. The other is the inability to utilize sugar as has been shown by A. T. Ringer (1), Lusk (2), Nash and Benedict (3) and others. It is unknown how this incapacity to metabolize sugar arises. Nash and Benedict brought forth a theory according to which a stable union between the "carbohydrate-receptors" and phlorhizin is formed with the result that the sugar molecules can no longer enter into the hypothetical combination prerequisite to their combustion. M. Ringer (4) in a recent paper on the influence of insulin on phlorhizin diabetes suggested the possibility that phlorhizin affects the pancreas by temporarily abolishing its internal secretion. Such an action of phlorhizin would, of course, account for the syndrome of phlorhizin diabetes. From Ringer's own paper and also from papers by Nash (5), C. F. Cori (6), Colwell (7) it may be seen that insulin is not a true antagonist of phlorhizin. This might be due to the fact that insulin does not antagonize the effect of phlorhizin on the kidneys. It was thought that by determining the insulin content of the pancreas of animals, completely poisoned with phlorhizin, some further insight might be gained into this problem. Since the results obtained did not point to an inhibition of insulin production by phlorhizin, an attempt was made to determine the insulin content of other tissues. The possibility was taken into consideration that the tissues, in spite of a normal production of insulin by the pancreas might be rendered unable to absorb insulin during phlorhizin poisoning. As will be seen from the experimental data, no significant difference in the insulin content of tissues of phlorhizinized and control animals could be detected.

EXPERIMENTAL. Best, Scott and Banting (8), Best and Scott (9), Best, Smith and Scott (10), Ashby (11), Ivy and Fisher (12) have shown that organs of mammals other than the pancreas contain appreciable amounts of insulin. The methods used for the preparation were very similar to those in use for the preparation of insulin from the pancreas. In the fol-

lowing experiments the Doisy, Somogyi and Shaffer method (13) was used for the preparation of insulin from pancreas, liver, skeletal muscles and blood. The slight modifications which had to be applied on account of the small amounts of insulin available in single organs other than pancreas, did not alter the process essentially.

The figures obtained for the insulin content of these tissues are by no means to be taken as standard values, but since in all experiments exactly the same technique was used they have a comparative value. Four cats were used. They were starved for 8 days. During the last 3 days two of the cats received one injection every 12 hours of 1 gram phlorhizin dissolved in oil. The urine of the last 24 hours was collected and the D:N ratio determined in order to make sure that a complete phlorhizin poisoning had been established. The cats were killed by bleeding through the carotid artery. A small quantity of ether was used during the preparation of the vessel. The pancreas, blood, liver and as much of the skeletal muscles as could easily be obtained were immediately worked up for insulin. After 2 days a crude solution of insulin was obtained, which was assayed on mice. Another test animal could not be employed since naturally only traces of insulin were extracted from organs other than the pancreas of a single cat. The fall in blood sugar and not the onset of convulsion in the mice was used as base for the assay. The mice were killed by decapitation one hour after the insulin injection, the blood was collected and the sugar content was determined in duplicates by the Hagedorn and Jensen (14) method. Krogh (15) has shown that the room temperature has to be considered when hypoglycemic symptoms in mice are used for assaying insulin. Since all experiments were performed during the summer and a room temperature below 24°C. was never recorded and since the blood sugar level and not the onset of convulsions was determined, special precautions to keep the mice at a higher and uniform temperature did not seem necessary. The yields of insulin that were obtained from the organs of two phlorhizinized and two control cats are expressed in mouse units since it did not seem advisable to calculate these small amounts of insulin in the commonly used rabbit units. The amount of insulin injected intraperitoneally which lowers the blood sugar of a mouse (previously starved for 24 hours, weighing 18 to 22 grams and kept not below 24.0°C.) in one hour to 0.038 to 0.044 per cent is called a mouse unit. This amount of insulin is probably very close to the mouse unit of Fraser (16) who used the onset of convulsions as base of the assay. Since all experiments were performed in the same way, one detailed protocol only is recorded below.

July 8, food taken away from cat 3.

July 13 to 15, injection twice daily of 1 gram phlorhizin dissolved in oil.

July 14 to 15, urine volume 174 cc. Total nitrogen 3.35 grams, sugar—9.10 grams, D:N ratio—2.71.

July 15, killed by bleeding through the carotid artery. Pancreas 3.88 grams, blood 50.0 grams, liver 61.8 grams, muscle 238.0 grams. All organs extracted according to Doisy's method. The precipitate obtained at the end of the process by the so-called isoelectric precipitation was dissolved in n/100 HCl with addition of 0.1 per cent tricresol.

Testing of "pancreas-insulin" dissolved in 4 cc. and injected intraperitoneally in the following amounts:

Mouse 1—0.1 cc. paralyzed after 20 minutes, convulsions after 40 minutes, recovered 5 minutes after a glucose injection.
Mouse 2—0.05 cc. paralyzed after 30 minutes, convulsions after 45 minutes, recovered 5 minutes after a glucose injection.
Mouse 3—0.04 cc. paralyzed after 45 minutes, in deep coma after 85 minutes, recovered 8 minutes after a glucose injection.
Mouse 4—0.04 cc. paralyzed after 32 minutes, convulsions after 55 minutes, recovered 4 minutes after a glucose injection.
Mouse 5—0.04 cc. paralyzed after 35 minutes, killed after one hour, blood sugar 0.037 per cent.
Mouse 6—0.03 cc. paralyzed after 45 minutes, comatose after 1 hour, fairly recovered after 3 hours.
Mouse 7—0.03 cc. paralyzed after 45 minutes, comatose after 120 minutes, recovered 10 minutes after a glucose injection.
Mouse 8—0.03 cc. paralyzed after 40 minutes, killed after 60 minutes, blood sugar 0.039 per cent.
Mouse 9—0.025 cc. paralyzed after 30 minutes. Began to recover after 2 hours.
Mouse 10—0.025 cc. paralyzed after 40 minutes, killed after 60 minutes, blood sugar 0.051 per cent.
Mice 11 and 12—0.02 cc. paralyzed after 60 minutes. Began to recover after 2 hours.
Mouse 13—0.02 cc. paralyzed after 50 minutes, killed after 1 hour, blood sugar 0.075 per cent.

Testing of "liver-insulin" dissolved in 3 cc. fluid and injected intraperitoneally in the following amounts:

Mice 14 and 15—0.5 cc. very weak after 30 minutes, in comatose state when killed after 1 hour. Blood sugar 0.045 per cent and 0.043 per cent respectively.
Mouse 16—0.6 cc. very weak after 25 minutes, comatose when killed after 1 hour. Blood sugar 0.05 per cent.

Testing of "blood-insulin" dissolved in 2.5 cc. and injected intraperitoneally in the following amounts:

Mouse 17—0.5 cc. deeply paralyzed after 15 minutes, in comatose state when killed after 1 hour. Blood sugar 0.029 per cent.
Mouse 18—0.4 cc. paralyzed after 30 minutes, comatose when killed after 1 hour. Blood sugar 0.032 per cent.
Mouse 19—0.3 cc. paralyzed after 35 minutes, comatose when killed after 1 hour. Blood sugar 0.033 per cent.
Mouse 20—0.25 cc. slightly paralyzed after 1 hour. Began to recover after 2½ hours.
Mouse 21—0.2 cc. slightly paralyzed when killed after 1 hour. Blood sugar 0.068 per cent.

Testing of "muscle-insulin" dissolved in 4 cc. and injected intraperitoneally in the following amounts:

Mice 22 and 23—0.3 cc. paralyzed when killed after 1 hour. Blood sugar 0.05 and 0.048 per cent respectively.
Mouse 24—0.4 cc. paralyzed after 1 hour. Began to recover after 3 hours.

The following table gives a summary of the yields of insulin obtained from the organs of two control and two phlorhizinized cats.

It was thought advisable to gain some data about the quantity of insulin that could be recovered on addition of a small amount of insulin to minced tissues of the body. By such a procedure nothing is learned about the actual extraction of insulin from the cells which undoubtedly depends to a large extent on the degree to which the tissues have been minced. It can only serve to ascertain whether insulin is lost during the

TABLE 1

The insulin content of the organs of control cats and of cats poisoned with phlorhizin

	MOUSE UNITS PER GRAM TISSUE			
	Phlorhizinized cats		Control cats	
	Cat 1	Cat 2	Cat 3	Cat 4
Pancreas.....	26	40	33	94
Liver.....	0.1	0.13	0.13	0.08
Blood.....	0.1	0.2	0.04	0.04
Muscle.....	Traces (0.04)	Traces (0.034)	Traces (0.007)	Traces (0.024)

subsequent stages of the preparation. From the following data it may be seen that only a small amount of insulin is lost during the precipitation with ammonium sulfate and the subsequent isoelectric precipitation of the Doisy method, even when very small quantities of insulin are added to a minced liver of a guinea pig. Two-hundredths cubic centimeter iletin (U20) were added in two experiments to the minced livers of guinea pigs. The livers weighed 21.3 and 18.2 respectively. The Doisy process was then followed and the final isoelectric precipitate was taken up in 3 cc. of fluid. For control purposes 0.02 cc. iletin from the same ampule was also made up to 3 cc. with saline and this solution served as standard. The solutions were tested in mice and the animals were killed one hour after the injection and the blood sugar determined.

Discussion. The insulin content of the pancreas of two completely phlorhizinized and two control cats has been determined. The values for both phlorhizinized and one control animal fell within the same range. The pancreas of the other control contained markedly more insulin. Since, on the other hand, the pancreas of one phlorhizinized cat contained actually more insulin than the pancreas of one control cat, an appreciable

reduction of the insulin content of the pancreas could not be detected during phlorhizin poisoning.

Practically equal amounts of insulin were found in the livers of phlorhizinized and control cats. The blood of the phlorhizinized cats contained more insulin than that of the control cats. A larger number of experiments would be necessary to establish whether this is a merely accidental finding or has some definite bearing. The skeletal muscles contained only traces of insulin and no difference between phlorhizinized and control cats could be detected.

TABLE 2

ORIGINAL ILETIN 0.02 CC. DILUTED TO 3 CC.		ILETIN, RECOVERED ON ADDITION OF 0.02 CC. TO LIVER TISSUE, DISSOLVED IN 3 CC.	
Injected	Blood sugar per 100 cc.	Injected	Blood sugar per 100 cc.
cc.	*gram*	I	
0.07	* 0.064	*cc.*	*gram*
0.1	0.063	0.1	0.085
0.1	0.057	0.11	0.070
0.15	0.061	0.3	0.052
0.15	0.054	0.3	0.060
0.15	0.036	0.4	0.059
0.2	0.048		
0.2	0.034	II	
0.2	0.046		
0.2	0.039	0.15	0.049
0.3	0.030*	0.2	0.039
0.3	0.057†	0.2	0.047
		0.25	0.046
		0.3	0.039
		0.3	0.038*

* Convulsions.

† Vehement convulsions.

CONCLUSION

The insulin content of the pancreas, liver, skeletal muscles and blood of completely phlorhizinized and starved cats has been compared with the insulin content of normal starved cats. No appreciable diminution of the insulin content of the organs of phlorhizinized animals could be detected.

While this paper was in press there appeared a communication by Nash and Benedict (*Journ. Biol. Chem.*, 1924, lxi, 423). These authors also found no lack of insulin in the pancreas of completely phlorhizinized dogs.

BIBLIOGRAPHY

(1) RINGER: Journ. Biol. Chem., 1912, xii, 431.
(2) LUSK: Journ. Biol. Chem., 1915, xx, 598.
(3) NASH AND BENEDICT: Journ. Biol. Chem., 1923, lv, 757.
(4) RINGER: Journ. Biol. Chem., 1923, lviii, 483.
(5) NASH: Journ. Biol. Chem., 1923, lviii, 453.
(6) CORI: Journ. Pharm. Exper. Therap., 1924, xxiii, 99.
(7) COLWELL: Journ. Biol. Chem., 1924, lxi, 289.
(8) BEST, SCOTT AND BANTING: Trans. Roy. Soc., Canada, May 1923, Sect. v.
(9) BEST AND SCOTT: Journ. Amer. Med. Assoc., 1923, lxxxi, 382.
(10) BEST, SMITH AND SCOTT: Journ. Biol. Chem., 1924, lix; Proc., xxx, This Journ., 1924, lxviii, 161.
(11) ASHBY: This Journal, 1924, lxvii, 77.
(12) IVY AND FISHER: This Journal, 1924, lxvii, 445.
(13) DOISY, SOMOGYI AND SHAFFER: Journ. Biol. Chem., 1923, lv, xxxi.
(14) HAGEDORN AND JENSEN: Biochem. Zeitschr., 1923, cxxxv, 46.
(15) KROGH: quoted from MACLEOD, Physiol. Reviews, 1924, iv, 36.
(16) FRASER; Journ. Lab. Clin. Med., 1923, viii, 425.

RESPIRATORY CHANGES DURING AND AFTER A PERIOD OF ANOXEMIA

EDWARD C. SCHNEIDER, DOROTHY TRUESDELL AND ROBERT W. CLARKE

From The School of Aviation Medicine, Mitchel Field, Long Island, New York

Received for publication November 29, 1924

Among people interested in aviation two remarks are occasionally heard that have made it seem worth while to go a little more fully than we have heretofore into the respiratory reactions of anoxemia. The first of these is the opinion that an aviator should be able to determine when he needs oxygen from his feeling of breathlessness, and a comparable statement in physiology that with a rapid reduction in the oxygen tension of the inhaled air there occurs a quite noticeable increase in the depth and frequency of breathing. The second is the report that the aviator breathes subnormally after a flight to a high altitude. It is, therefore, our purpose to analyze the respiratory changes during two rates of producing anoxemia in a low pressure chamber, and also to examine the changes that immediately follow a period of anoxemia. The first rate at which the anoxemia was developed corresponds reasonably well with the rate an airplane may ascend; while the second is far more rapid than any airplane has yet attained.

THE RESPIRATORY CHANGES DURING ANOXEMIA. Boycott and Haldane (2) have shown that a sudden decrease in the tension of oxygen in the inspired air causes an increase in the depth and frequency of breathing which washes a considerable amount of carbon dioxide from the blood. The evidence of washing out was obtained in the lowered tension of carbon dioxide in the lungs. A detailed study of the alveolar air changes in anoxemia has been reported by Lutz and Schneider (10). In 24 subjects taken to a barometric pressure of 350 mm. (20,000 feet) in a low pressure chamber, at a rate equivalent to an altitude ascent of 1000 feet a minute, the average alveolar carbon dioxide tension fell from 39.6 mm. at sea level to 30 mm. at 350 mm. The decrease in the alveolar carbon dioxide tension was already present in some cases at a barometric pressure of 650 mm. (4000 feet), at which it averaged about 2 mm. This indicates that in some persons the breathing begins to augment at a rather low altitude, 4000 feet. A further study of the alveolar air tension was made during a stay under a constant low barometric pressure. The

lowest level of the alveolar carbon dioxide tension is ordinarily not reached during the time the barometric pressure is being lowered, but occurs 5 to 10 minutes after the constant low level of atmospheric oxygen pressure is established, after which for awhile the alveolar carbon dioxide tension remains constant. Later, in a majority of cases, in those who react well to the anoxemia, the alveolar carbon dioxide tension rises slowly. However, if the subject does not tolerate the anoxemia well, the alveolar carbon dioxide tension continues to fall steadily during the period of constantly maintained low oxygen. Between these two classes of persons stands a third in which the alveolar carbon dioxide tension will, for a few minutes, continue to decrease after the low oxygen level is established and then remain constant during the remainder of the period.

Lutz and Schneider also found, as was anticipated, that the minute-volume of breathing undergoes changes that correspond to those of the alveolar air. During the period of reduction of barometric pressure an increase in the ventilation of the lungs begins between 656 and 605 mm. (4000 and 6000 feet) and then augments gradually and steadily thereafter. When a constant pressure is maintained during the subsequent 30 to 120 minutes, the maximum ventilation, in most persons, occurs within 10 minutes after the barometric pressure of 350 mm. is reached. Following this period there is a distinct falling off in the minute-volume of breathing.

We now have further data on the volume and rate of breathing and, in addition, data on the carbon dioxide output during similar exposures to anoxemia. The carbon dioxide output was determined for consecutive intervals of 5 minutes during the time the barometric pressure was being reduced to 350 mm. (20,000 feet); while a uniform low pressure was maintained; and during a post-anoxemic period of variable duration. For the collection of the expired air the Douglas bag was used and all analyses were made in duplicate or triplicate with the Haldane gas analysis apparatus. The reduction in barometric pressure was made at the rate of 21 mm. a minute, approximately equivalent to an altitude ascent of 1000 feet a minute. In a few experiments the barometric pressure was lowered at the rate of 137 to 145 mm. per minute, equivalent to an ascent of approximately 6500 feet per minute. The after-effects of anoxemia were, in some cases, observed during administration of pure oxygen; and, in others, during the period of restoration to normal or sea-level pressure and after the reëstablishment of the normal barometric pressure.

The changes when the barometric pressure was reduced at a rate comparable to an ascent of a 1000 feet a minute. The data for 2 series of experiments are given in tables 1 and 2. From these it is evident that the increase in the minute-volume and the frequency of breathing occurred so gradually and to so small an extent that the subject of experimenta-

TABLE 1

Respiration during anoxemia and oxygen inhalation. Decompression to 350 mm. in 20 minutes

	760 MM.	515 to 425 MM.	425 TO 350 MM.	TIME AT 350 MM.					BREATHING PURE OXYGEN			
				0–5 minutes	6–10 minutes	11–15 minutes	16–20 minutes	40–45 minutes	0–5 minutes	6–10 minutes	11–15 minutes	16–20 minutes
R. W. C.												
Minute-volume, liters	7.1	7.6	10.2	8.8	8.7	8.1	8.7	9.3	6.0	4.2	5.2	5.7
Breaths per minute	13.4	16.6	17	12.8	13	10.4	11.2	17	13.2	14.4	15.6	15.0
Carbon dioxide output, cc	215	221	277	234	226	199	221	173	129	95	121	148
Carbon dioxide, cc. per liter ventilation	30.3	29.1	27.2	26.6	26.0	24.6	25.4	18.6	21.5	22.6	23.3	26.0
D. T.												
Minute-volume, liters	6.3	6.9	8.0	9.0	10.6	11.9	13.0	11.1	10.4	7.5	5.3	5.3
Breaths per minute	14.1	15.2	16.2	18.6	20.4	20.0	18.8	19.4	18.6	17.0	14.2	16
Carbon dioxide output, cc	161	174	188	198	200	212	215	191	158	118	90	97
Carbon dioxide, cc. per liter ventilation	25.6	25.2	23.5	22.0	18.9	17.8	16.1	17.2	15.2	15.7	17.0	18.3
L. M. T.												
Minute-volume, liters	8.1	9.4	10.3	11.8	10.8	9.8	9.0	9.3	7.8	7.5	6.4	6.6
Breaths per minute	12.4	14.6	14.2	15.6	14.8	16.0	14.0	14.4	13.6	13.8	12.0	14.8
Carbon dioxide output, cc	283	315	302	376	323	289	276	266	252	231	188	181
Carbon dioxide, cc. per liter ventilation	34.9	35.0	29.3	31.9	30.0	29.5	30.6	28.6	32.3	30.8	29.3	27.4
W. C. M.												
Minute-volume, liters	6.5	7.5	9.1	8.7	8.2	8.6	8.0	8.8	6.4	4.9	4.1	4.6
Breaths per minute	8.6	8.8	8.4	9.8	9.2	9.2	10.4	9.4	10.8	10.4	11.0	12.0
Carbon dioxide output, cc	209	257	304	252	234	240	227	207	172	137	128	133
Carbon dioxide, cc. per liter ventilation	32.2	34.3	33.6	29.0	28.5	28.0	28.4	23.5	26.9	28.0	31.1	28.9

tion could scarcely have been aware of the changes unless he had given close attention to them. The maximum increase in minute-volume during the 20 minutes of decompressing was only 3.1 liters. During the period of maintained pressure the respiratory ventilation usually showed a further increase, the greatest increase amounted to 6.7 liters. This was reached almost 20 minutes after the level of pressure was established and required at least 35 minutes to be developed. The increase in frequency ranged between 1.8 and 6.3 breaths per minute. This change also occurred gradually and in the case that gave the maximum increase did not become maximal until 25 minutes of the experimental period had passed. It is our experience that ordinarily even the experienced physiologist does not notice the change obtained by this degree and rate of developing anoxemia.

There occurs, as the alveolar air and minute-volume studies indicated, an increase in the elimination of carbon dioxide early in the period of reduction of barometric pressure. It will be observed that in all but one of the 8 experiments recorded in tables 1 and 2, the output of carbon dioxide was already increased at the time of the first collection of respired air, which was made during the 5 minutes as the barometric pressure was lowered from 515 to 425 mm. (altitudes 10,000 to 15,000 feet). The increase in output then ranged from 2.8 to 29.1 per cent.

While a blowing off of carbon dioxide begins early in the period of decompression, as early as at 650 mm. (4000 feet), it does not, as a rule, become maximal during the time the barometric pressure is being reduced. Among the 8 cases there were 3 that gave the maximum elimination during the period of decompression, while the others gave it after the pressure of 350 mm. had been reached and maintained for awhile.

Among the latter the maximum output occurred in 2 instances during the first 5 minutes spent under the constant low barometric pressure, in another between the 11th and 15th minutes, in one between the 16th and 20th minutes, and in still another not until between the 21st and 25th minutes. The maximum increase obtained in the elimination of carbon dioxide at 350 mm. amounted to 52.7 per cent.

The process of blowing off the excess of carbon dioxide is of variable duration. In some persons it was completed within from 10 to 20 minutes, but in others not within the 45 minutes of our short time experiments. In the 2 short time experiments on D. T., reported in tables 1 and 2, the process was not quite finished; but in a longer experiment, in which this subject was held over 4 hours at 380 mm. (see table 4, THIS JOURNAL, lxx, p. 283), the process was complete within 4 hours.

In the early stages of anoxemia the production of carbon dioxide fails to keep pace with the elimination. Eventually, because of excessive elimination, the carbon dioxide content of the blood becomes so depleted

that for awhile the minute-output is subnormal. Another factor that accounts for the period of subnormal output of carbon dioxide is a temporary decrease in the rate of oxidation (11) in the body. In our experience the minute-output of carbon dioxide has sometimes become subnormal as early as the end of 15 minutes spent under the uniform low barometric pressure. In 3 of 8 experiments the subnormal phase was not reached within 45 minutes. In 4 longer experiments, which we reported in a former paper (11), the subnormal phase persisted for some time and was then followed by a period of increasing output. In L. T., who was held 5½ hours at 380 mm., the carbon dioxide output had, in the blowing off process, increased from the normal output of 231 cc. per minute to 244 cc. during the 10th minute at 350 mm. and to 260 cc. during the 25th minute. It then became subnormal and was 218 cc. at the end of 1.2 hours and 200 cc. at the end of 2.2 hours. Following this the output tended to return to normal and was nearly restored at the end of 3.2 hours, when it was 226 cc. In one experiment on R. W. C., which lasted 8 hours, and in which the blowing off stage was not studied, the carbon dioxide output became subnormal in about 15 minutes. His normal output per minute was 209 cc.; while it was 182 cc. the 27th minute, 185 cc. at 1.5 hours, 200 cc. at 4 hours, 214 cc. at 5.5 hours, 212 cc. at 6.5 hours and 197 cc. at 7.5 hours. In this case it appears that a brief period of blowing off of carbon dioxide was followed by a period of subnormal output of approximately 5 hours duration, after which the elimination of carbon dioxide once more returned to normal. In another experiment of 5.5 hours with R. W. C., in which symptoms of mountain sickness were manifest, the output of carbon dioxide for a second time became excessive after 3 hours.

The volume of carbon dioxide that is eliminated in excess of the normal output can be estimated from our data given in tables 1 and 2. R. W. C. in one experiment eliminated an excess of 490 cc. and in another approximately 1205 cc. L. M. T. in one experiment finished the blowing off process in 15 minutes after the barometric pressure of 350 mm. had been reached and eliminated an excess of 950 cc.; while in another experiment, in which the blowing off process was not completed, he eliminated an excess of 2415 cc. In 2 experiments on D. T. the excess of the output of carbon dioxide was approximately 1855 cc. and 1110 cc. respectively. In W. C. M. the excess amounted to 1290 cc. and in H. E. A. to 1015 cc.

Our data indicate that the supply of carbon dioxide in the blood is being further decreased even after the process of blowing off of carbon dioxide seems to be completed. The data for the minute-volume output of carbon dioxide indicate that the blowing off process is ordinarily completed within 15 to 25 minutes, but that occasionally approximately 4 hours are required, under the constant low level of the barometric pres-

TABLE 2

Respiratory changes during decompression

TABLE 2

Respiratory changes during decompression to 350 mm. and return to 760 mm.

	760 MM.	515 TO 425 MM.	425 TO 350 MM.	TIME AT 350 MM.					435 TO 515 MM.	630 TO 760 MM.	TIME AT 760 MM.		
				0–5 minutes	11–15 minutes	21–25 minutes	31–35 minutes	41–45 minutes			0–5 minutes	11–15 minutes	21–25 minutes
R. W. C.													
Minute-volume, liters	7.7	7.6	9.8	12.6	10.4	8.7	9.1	8.8	7.3	5.2	5.8	6.5	6.5
Breaths per minute	14.5	17.0	16.8	16.0	14.8	14.0	15.2	17.0	15.0	14.8	14.4	15.6	14.2
Carbon dioxide output, cc.	232	228	271	324	262	221	234	202	135	127	148	164	184
Carbon dioxide, cc. per liter ventilation	30.1	30.0	27.7	25.7	25.2	25.4	25.4	23.0	18.5	24.4	25.5	25.2	28.3
D. T.													
Minute-volume, liters	6.3	7.4	8.8	9.3	9.8	9.2	8.1	8.3	5.0	5.4	6.6	6.5	6.5
Breaths per minute	16.2	16.2	16.2	16.6	16.6	17.2	13.0	14.4	9.6	13.3	18.0	16.0	16.4
Carbon dioxide output, cc.	165	172	201	190	205	185	173	169	109	118	138	149	142
Carbon dioxide, cc. per liter ventilation	26.2	23.2	22.8	20.4	20.9	20.1	21.4	20.4	21.8	21.8	20.9	22.9	21.8
H. E. A.													
Minute-volume, liters	5.4	7.4	8.4	7.7	7.2	7.0			5.2	4.6	5.4	5.7	5.6
Breaths per minute	15.0	15.6	16.2	16.8	17.2	19.9			17.0	16.0	15.9	15.0	15.0
Carbon dioxide output, cc.	151	213	233	189	171	150			121	107	128	139	143
Carbon dioxide, cc. per liter ventilation	28.0	28.8	27.7	24.5	23.8	21.4			23.3	23.3	23.7	22.6	25.5

L.M.T.

	760 MM.	515 TO 425 MM.	425 TO 350 MM.	TIME AT 350 MM.						350 TO 425 MM.	425 TO 515 MM.	515 TO 630 MM.	630 TO 760 MM.	TIME AT 760 MM.			
				0–5 minutes	6–10 minutes	11–15 minutes	16–20 minutes	21–25 minutes	26–30 minutes					0–5 minutes	6–10 minutes	11–15 minutes	16–20 minutes
Minute-volume, liters	8.0	9.7	9.7	10.8	10.8	11.5	11.1	10.7	10.6	10.8	9.1	9.5	7.5	8.1	7.6	6.9	7.3
Breaths per minute	14.4	15.4	13.0	16.0	16.4	16.8	17.8	18.0	17.8	18.2	18.6	15.5	13.5	14.0	13.5	12.2	13.5
Carbon dioxide output, cc.	264	328	324	320	318	340	320	291	280	298	276	292	207	252	241	220	226
Carbon dioxide, cc. per liter ventilation	33.0	33.8	33.4	29.6	29.4	29.5	28.5	27.2	26.4	27.6	30.3	30.7	27.6	31.1	31.7	31.9	31.0

sure. However, it appears that for each liter of air breathed the amount of carbon dioxide eliminated becomes steadily less during the entire stay at a given low barometric pressure. This was the case in the short time experiments recorded in tables 1 and 2. With but one exception the output per liter of ventilation was less for the last test, made at the end of 45 minutes, than in the preceding determination. Thus in the experiment on R. W. C., in table 1, the amount of carbon dioxide eliminated per liter of air breathed was 30.3 cc. at 760 mm., 29.1 cc. at 515–425 mm., 27.2 cc. at 425–350 mm., 26.6 cc. during the first 5 minutes at 350 mm., 26.0 cc. during the second 5 minute-period, 24.6 cc. in the third, 25.4 cc. in the fourth, and 18.6 cc. at the 45th minute. The ratio falls rapidly during decompression and the first minutes at the constant pressure, and thereafter undergoes a very gradual further fall. In our longer experiments of from 4 to 8 hours' duration the same condition was found. Thus for R. W. C., in an 8 hour experiment, the carbon dioxide output per liter of ventilation was as follows: normal 29.4 cc., at 380 mm. in 10 minutes 25.4 cc., in 27 minutes 22.5 cc., in 1.5 hours 20.1 cc., in 4 hours 19.2 cc., in 5.5 hours 20.2 cc., in 6.5 hours 19.8 cc., and in 7.5 hours 17.3 cc. Just how much change in the carbon dioxide content of the blood this condition indicates is not clear; unfortunately we made no alveolar air analyses in any of these experiments.

The minute-volume of breathing, of course, does not run parallel with carbon dioxide output per minute, nor with the amount of carbon dioxide eliminated per liter of ventilation. In the short experiments the minute-volume of breathing increased early, then, after reaching a maximum, slightly decreased and eventually reached a level which, being still above normal, was maintained fairly evenly throughout the remainder of the 45 minutes. In the long experiments of from 5 to 8 hours, the minute-volume, after the uniform period, again rose slightly in the latter part of the stay in the low pressure chamber.

The response to a rapidly developed anoxemia. It has been maintained that when a deficiency of oxygen is produced gradually the initial increase in breathing is not as great as when the same degree of deficiency is produced rapidly. Our experience indicates that this is true only when extreme differences in time occur; as, for example, in the anoxemia of nitrogen inhalation and that resulting from the rebreathing of 52 liters of air. We give the data of 3 experiments in table 3 in which the barometric pressure was reduced to 350 mm. in 3 minutes. The subjects who served for these also served in experiments, recorded in tables 1 and 2, in which the same pressure was reached in 20 minutes. In the experiments on R. W. C. in which the pressure was reduced in 3 minutes, the minute-volume of breathing increased from a normal of 7 to 10.9 liters, 55.7 per cent; while in one of two experiments, with a 20 minute

reduction period, the increase was from 7.7 to 12.6 liters, 63.6 per cent, and in the other from 7.1 to 10.2 liters, 43.6 per cent, during decompression. The carbon dioxide output rose, in the 3-minute reduction period, from 212 to 291 cc., 37.2 per cent; and in a 20-minute reduction period, from 232 to 324 cc., 39.7 per cent. In L. M. T. the minute-volume and carbon dioxide output were affected in practically equal degree under the two speeds of reduction in pressure. In D. T. the minute-volume and

TABLE 3

Respiration at 350 mm. Decompression in 3 minutes

	760 MM.	TIME SPENT AT 350 MM.							
		0–4 minutes	5–9 minutes	10–14 minutes	15–19 minutes	20–24 minutes	25–29 minutes	30–34 minutes	35–39 minutes
R. W. C.									
Minute-volume in liters	7.0	10.9	10.5	11.0	11.1	9.3	9.7	8.6	9.2
Breaths per minute	13.8	15.2	16.4	17.0	13.4	14.2	16.4	13.8	15.2
Carbon dioxide output, cc.	212	291	260	259	236	222	212	197	205
Carbon dioxide, cc. per liter ventilation	30.3	26.7	24.8	23.5	21.2	23.5	21.9	22.9	22.3
D. T.									
Minute-volume, liters	7.4	10.2	11.8	9.2	10.2	9.7	8.5	8.8	
Breaths per minute	18.1	22.4	20.6	19.1	20.0	17.6	12.0	13.8	
Carbon dioxide output, cc.	163	263	194	166	176	185	153	158	
Carbon dioxide, cc. per liter ventilation	22.0	25.8	16.4	18.0	17.2	19.1	17.9	17.9	
L. M. T.									
Minute-volume, liters	8.7	10.3	10.3	8.8	11.7	10.2	11.7	10.1	11.4
Breaths per minute	16.1	15.2	14.4	16.6	14.8	16.4	14.0	16.6	15.2
Carbon dioxide output, cc.	249	299	283	224	328	273	320	267	299
Carbon dioxide, cc. per liter ventilation	28.6	29.0	27.5	25.5	28.0	26.8	27.3	26.1	26.2

the carbon dioxide output per minute increased most in the experiment in which the reduction in pressure was made in 3 minutes. The excess of carbon dioxide washed out in 40 minutes, as the result of rapid and slow reduction of pressure, was for R. W. C. 1040 and 1205 cc. respectively; for D. T. 845 and 1110 cc.; and for L. M. T. 1630 and 2275 cc. It is evident that the differences between the rapid and slow reduction in pressure are not marked nor are they uniformly in one direction.

It may now be worth while to ascertain how well the respiratory reactions of anoxemia conform to the several theories of respiration. Hal-

dane (5) and his collaborators have emphasized the close relationship between the acidity of the blood and respiratory activity. They believe that lack of oxygen lowers the threshold of stimulation of the respiratory center to the carbon dioxide or hydrogen ion content of the arterial blood. Haldane, Kellas and Kennaway (6) find the first effect of anoxemia to be a washing out of a considerable excess of preformed carbon dioxide from the blood, after which the breathing settles down to correspond with the lowered threshold of alveolar carbon dioxide pressure. They hold that it is the diminution in the hydrogen ion content of the blood that checks the increased breathing. It seems to us that the delayed maximal effect on the minute-volume of breathing, and output of carbon dioxide during the early part of the period of exposure to anoxemia, does not find adequate explanation in this theory. That the alkalosis, which results from the blowing off of carbon dioxide, may account for the quieting of breathing is a possible explanation of the later reduction in the pulmonary ventilation; but it fails to account for the fact that in longer continued anoxemia, the breathing may once more begin to increase and later even exceeds the maximal volume experienced during the earlier part of the period of anoxemia.

Lindhard (9), and Hasselbalch (7) have concluded that the excitability of the respiratory center toward the stimulus of carbon dioxide or the hydrogen ion concentration of the blood is increased by a lack of oxygen in the inspired air. In general it is thought that the excitability varies in inverse proportion to the oxygen tension. This theory likewise does not readily account for the comparatively slow increase in the minute-volume of breathing. Furthermore, in many instances the alveolar carbon dioxide tension rises slightly during the period of a constantly maintained barometric pressure as the alveolar oxygen remains on a level or decreases slightly, while at the same time the minute-volume of breathing is somewhat falling off. When later the breathing again begins to increase in volume the alveolar carbon dioxide tension is then decreasing and the oxygen tension holding steady or rising slightly.

Gesell (3) has suggested that changes in the hydrogen ion concentration of the respiratory center, rather than of the blood, constitute the determining factor in respiratory control. Because the supply of oxygen determines the amount of lactic acid and carbon dioxide formed in living tissues, he believes that it is the normal and indirect regulator of pulmonary ventilation. By virtue of its own metabolism and its sensitivity to changes in its own hydrogen ion concentration, the center is sensitive to minute changes in its own oxidations. As regards the influence of the composition of the blood it is stated that neither the number of hydrogen ions brought to the center nor the concentration of the hydrogen ions of the arterial blood determines the extent of ventilation, but rather

the efficiency of the blood as a carrier of the hydrogen ions away from the center. With a lack of oxygen there occurs not only a diminution of oxidation, with a relative increase in acid, but also a failure in the coördination of the dual function of the hemoglobin. The lack of the usual reduction of oxyhemoglobin fails to provide the alkali which ordinarily unites with carbon dioxide and lactic acid to neutralize them. Consequently acids accumulate in the center. This theory provides the best explanation for the respiratory variations of the type of anoxemia we have studied. According to it the early slow increase in the minute-volume of breathing is accounted for by a gradual accumulation of carbon dioxide and lactic acid in the center, because of a reduction in the rate of oxidation that occurs when the oxygen tension of the arterial blood is lowered and by the failure of the blood to provide the usual amount of alkali for removal of the acids. The later quieting of the breathing is probably the result of the washing out of carbon dioxide from the blood, whereby the carbon dioxide content of the blood is so lowered that the carbon dioxide diffusion gradient from the center to the blood is increased sufficiently for the respiratory center to be relieved of some of its excess of carbon dioxide. The later increase in the breathing that sometimes has been observed during longer exposures to a low barometric pressure finds an explanation in the observation of Koehler, Brunquist and Lowenhart (8). In agreement with Henderson and Haggard (4), they find that a very definite increase in the proportion of alkali of the blood, an alkalosis, results from the rapid elimination of carbon dioxide by the lungs during the early part of the anoxemia; but they further find that when the anoxemia is continued for several hours the hydrogen ion content returns to normal and continues to decrease until an acidosis results. They believe that the production of fixed acids begins at once, but does not keep pace with the loss of carbon dioxide from the blood. Later the acid production more than compensates for the loss of carbon dioxide. When this occurs the elimination of acids from the respiratory center will be again retarded and the breathing will once more increase, as it did in several of our experiments of 5 to 8 hours. This same steady increase of acid in the blood accounts for our observation that the amount of carbon dioxide per a liter of ventilation steadily decreases during the period of anoxemia.

THE AFTER-EFFECTS OF ANOXEMIA. The after-effects on the respiration of a stay under a low partial pressure of oxygen are determined by the length of the time spent there, the extent to which carbon dioxide has been blown off, and the change in the proportion of alkali in the blood. Following short periods of anoxemia an alkalosis of the blood is present, hence when the anoxemia is relieved the respiration should at once be modified so as to permit an accumulation of carbon dioxide in

sufficient amount to offset the alkalosis. Observations by Lutz and Schneider (10) on the alveolar air indicate that this may be accomplished fairly quickly. After a stay of from 30 to 90 minutes in a low pressure chamber at 425 mm., in 4 out of 5 subjects the alveolar carbon dioxide tension was back to normal within 20 minutes; but in only 4 out of 9 subjects was the return made in the same time after a stay at 380 mm. During the post-anoxemic period the body makes up the loss in carbon dioxide and may even recall alkali into the blood.

In our study of the after-effects of anoxemia, we have followed the changes in breathing during the administration of oxygen to subjects who had been at a barometric pressure of 350 mm. for 45 minutes; and also, in another set of experiments, the changes were followed as the normal barometric pressure was being restored and for 25 minutes thereafter. The data for oxygen administration are given in table 1. The inhalation of oxygen immediately caused a reduction in the minute-volume of breathing and in the output of carbon dioxide. In some instances the frequency of breathing was also reduced. The full effect, however, did not occur at once, as might have been expected if the reaction is due only to a return to normal oxidation in the respiratory center; but developed rather slowly. Thus the minute-volume of breathing of R. W. C. fell from 9.3 liters to an average of 6.0 liters during the first 5 minutes of oxygen breathing, to 4.2 liters during the interval from 5 to 10 minutes; then rose to 5.2 liters from the 11th to 15th minutes and to 5.7 liters from the 16th to 20th minutes. The delay in reaching the full quieting effect was even more marked in the experiment with D. T. in whom the volume of breathing fell from 11.1 liters to an average of 10.4 liters the first 5 minutes, to 7.5 liters from the 5th to the 10th minutes and to 5.3 liters during the 11th to 20th minutes. The minute-volume of breathing came back to normal in only one experiment out of eight.

The carbon dioxide output also falls off immediately when anoxemia is relieved, is slow in showing the maximum effect, and remains subnormal for quite awhile. During oxygen inhalation the output of carbon dioxide per minute for R. W. C. fell from an average of 173 to 129 cc. in the first and to 95 cc. in the second 5-minute period. This was followed by a rise to 121 cc. in the third and 148 cc. in the fourth period. His output was still below normal, 215 cc. per minute, at the end of 20 minutes. In the case of D. T. oxygen administration caused the output of carbon dioxide to drop from an average of 191 cc. to 158 cc. per minute in the first, to 118 cc. in the second and to 90 cc. in the third 5-minute period; after which the elimination increased to 97 cc. in the fourth period. The normal output was 161 cc., hence a very marked retention of carbon dioxide was still in progress at the end of 20 minutes. L. M. T. showed a fall in his output from 266 cc. to 252 cc. per minute during the first,

to 231 cc. in the second, to 188 cc. in the third, and to 181 cc. in the fourth 5-minute period. The full effect that led to the maximum retention of carbon dioxide was, therefore, delayed at least 15 minutes. He too was still at the height of his retention process at the time the experiment was terminated. W. C. M. showed a similar retention which fell from 207 cc. to 172 cc. per minute in the first period. The output was 137 cc. in the second, 128 cc. in the third, and 133 cc. in the fourth period. His normal output of carbon dioxide before the development of anoxemia was 209 cc. per minute.

In our study of the retention of carbon dioxide caused by increasing the barometric pressure from 350 to 760 mm. it was found that 3 cases began to conserve carbon dioxide as the pressure rose from 425 to 515 mm. (15,000 to 10,000 feet). For these data see table 2. Some retention undoubtedly began during the preceding 5 minutes as the pressure rose from 350 to 426 mm. In R. W. C. the minute-output of carbon dioxide fell from 202 cc. to 135 cc. at 515 mm., and in the 5-minute period from 625 to 760 mm. to 127 cc. During the next 25 minutes the output gradually increased to 184 cc.; but was not wholly restored to the normal amount, 232 cc., even after he had been at 760 mm. for 25 minutes, or 45 minutes from the time the return to the sea level pressure was begun. In the case of D. T. the carbon dioxide output fell from 169 cc. to 109 cc. as the barometric pressure rose from 425 to 515 mm.; the output was slightly larger as the pressure was being further increased from 625 to 760 mm.; and steadily increased during the next 25 minutes, at which time it was still 14 per cent below normal. H. E. A. gave a similar response, but the carbon dioxide output was more nearly back to normal at the end of 25 minutes after normal atmospheric pressure had been reëstablished than in any other case. Her normal output was 151 cc. per minute and for the last determination was 143 cc.

An attempt was made to follow the retention process on L. M. T. more carefully than in other cases; but, owing to the fact that he did not respond as quickly as in other experiments, the results add but little to the above observations. He did not begin to retain carbon dioxide until the barometric pressure had been increased to 625 mm., then the output suddenly fell from 292 cc. to 207 cc. per minute. In the following 20 minutes the output per minute, as was determined by 5-minute periods, was 252, 241, 220 and 226 cc. respectively. His normal output was 264 cc. So the carbon dioxide output per minute was still 14.4 per cent below normal at the end of 20 minutes spent at the sea-level pressure.

That the carbon dioxide content of the blood had not returned to normal during the after-period of 20 to 45 minutes in the subjects used in 2 sets of experiments is also evidenced in the proportion of carbon dioxide eliminated per liter of pulmonary ventilation. Although the proportion

of carbon dioxide per liter of ventilation increased at once, as the minute-volume of breathing declined, on relief from anoxemia; yet the two series of changes were not proportionate as the after-period progressed. In general the carbon dioxide elimination per liter of ventilation rose gradually and steadily, but was not back to normal in any case at the time observations were discontinued. In R. W. C., after a stay of 45 minutes at a barometric pressure of 350 mm., the minute-volume of breathing was 9.3 liters and the carbon dioxide output per a liter of breathing was 18.6 cc. (see table 1). During the first 5 minutes in which oxygen was breathed the minute-volume dropped to 6 liters and the output of carbon dioxide rose to 21.5 cc. per liter of ventilation; during the next 5 minutes the minute volume dropped to 4.2 liter, while the output of carbon dioxide rose to 22.6 cc.; and during the next 5 minutes the minute-volume rose to 5.2 liter, while the carbon dioxide output continued to rise to 23.3 cc. At the end of 20 minutes of oxygen inhalation the minute-volume of breathing was 5.7 liters and the carbon dioxide output 26 cc. Prior to being subjected to the low barometric pressure the carbon dioxide output per liter of pulmonary ventilation of R. W. C. was 30.3 cc. Therefore, the proportion of carbon dioxide in the exhaled air was still 14.2 per cent below normal after 20 minutes of breathing oxygen. In another experiment on R. W. C. (see table 2), after a stay of 45 minutes under a barometric pressure of 350 mm., the pressure was gradually increased to 760 mm. in 20 minutes. During the 5 minutes that the pressure was increased from 425 to 515 mm. (altitude 15,000 to 10,000 feet) the pulmonary ventilation averaged 7.3 liters per minute and the output of carbon dioxide per liter of ventilation 18.5 cc. As the pressure was lowered from 630 to 760 mm. (5000 feet to sea-level), the minute-volume of breathing averaged only 5.2 liters and the output of carbon dioxide 24.4 cc. per liter of ventilation. During the first 5 minutes spent at 760 mm. the minute-volume of breathing averaged 5.8 liter and the carbon dioxide output 25.5 cc. After 25 minutes had been spent at 760 mm. the minute-volume was 6.5 liters and the carbon dioxide output per liter of ventilation was 28.3 cc. His normal output of carbon dioxide per liter of ventilation was 30.1 cc. So in 45 minutes from the time that the anoxemia began to be relieved the output of carbon dioxide per liter of ventilation was still about 6 per cent below normal. These 2 experiments are quite typical of others reported in tables 1 and 2. After 20 minutes of oxygen administration the carbon dioxide output per liter of breathing in all cases ranged from 11.4 to 28.5 per cent below normal and in the cases in which the anoxemia was relieved by restoring the sea-level barometric pressure, after 45 minutes, the output still ranged from 6 to 16.8 per cent below normal. Apparently the acid-base balance of the blood had not then been restored to normal.

The slow appearance of the maximal quieting effect in the post-anoxemic period appears to conform to Gesell's theory of the control of breathing. Observations by Barr and Himwich (1) on the escape of acids from muscles after exercise indicate that once acids have accumulated in tissues they are rather slowly eliminated when conditions again become favorable for removal. The immediate quieting of breathing in our experiments is the result of the restoration of the metabolism of the respiratory center to normal, while the full quieting effect appears when the blood has removed the excess of acid. The subsequent slow return of the breathing to normal is dependent on the rate of restoration of the acid-base balance of the blood.

SUMMARY

When men are placed under a low barometric pressure at the rate of 21 mm. per minute (comparable to an ascent of 1000 feet) and then held steadily at 380 or 350 mm. (18,000 and 20,000 feet), the minute-volume of breathing begins to augment at about 650 mm. (4000 feet) and thereafter for a while continues to steadily increase. The maximum volume is sometimes obtained during the period of decompression, but ordinarily not until from 5 to 25 minutes have been spent under the constant pressure. Subsequently the volume of breathing falls off slightly and is then maintained at the new level for several hours. Still later the pulmonary ventilation is again likely to augment.

The changes in the rate and volume of breathing occur so gradually that the subject of experimentation remains unaware of them.

The elimination of carbon dioxide, of course, also begins to increase during the period of decompression and becomes maximal at about the time of largest pulmonary ventilation. However, the production of carbon dioxide fails to keep pace with elimination. Hence, eventually the output per minute becomes subnormal. The subnormal output is not only due to the excessive elimination but also to a reduction in the rate of oxidation in the body. The output of carbon dioxide may become subnormal as soon as 15 minutes have been spent under the constant low barometric pressure but sometimes not within 45 minutes.

The proportion of carbon dioxide eliminated per liter of air breathed steadily decreases throughout periods of anoxemia of 45 minutes to 8 hours. This, it is believed, indicates that the carbon dioxide content of the blood is steadily being depleted.

Reducing the barometric pressure in 3 and in 20 minutes to the same extent does not materially alter the rate and degree of respiratory response of anoxemia.

When anoxemia is relieved, either by the administration of oxygen or the restoration of the normal barometric pressure, an immediate reduc-

tion in the minute-volume of breathing and the output of carbon dioxide occurs. However, the full quieting effect does not occur for from 10 to 20 minutes. Subsequently the breathing rather slowly returns to normal.

The respiratory changes of anoxemia more nearly conform to Gesell's theory of the control of respiration than to other theories.

BIBLIOGRAPHY

(1) Barr and Himwich: Journ. Biol. Chem., 1923, lv, 525.
(2) Boycott and Haldane: Journ. Physiol., 1908, xxxvii, 355.
(3) Gesell: This Journal, 1923, lxvi, 5.
(4) Haggard and Henderson: Journ. Biol. Chem., 1919, xxxix, 163; 1920, xliii, 3, 15.
(5) Haldane: Respiration. Yale University Press, 1922.
(6) Haldane, Kellas and Kennaway: Journ. Physiol., 1919, liii, 181.
(7) Hasselbalch: Biochem. Zeitschr., 1912, xxxviii, 77.
(8) Koehler, Brunquist and Loevenhart: This Journal, 1923, lxiii, 404.
(9) Lindhard: Journ. Physiol., 1911, xlii, 336.
(10) Lutz and Schneider: This Journal, 1919, l, 280.
(11) Schneider, Truesdell and Clarke: This Journal, 1924, lxx, 283.

AGE AND SEX DIFFERENCES IN THE DAILY FOOD-INTAKE OF THE ALBINO RAT

GING H. WANG

From the Psychological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore

Received for publication October 22, 1924

In making studies on the activity of animals it is of prime importance to know that the animals are in good health and free from all diseases. Without this knowledge the results obtained are apt to be complicated by factors brought in by ill health. In all of the experiments carried on in this laboratory in the past few years we have used as an indicator of the condition of the animal, daily amount of food consumed and the weekly body weight. Any illness or disease shows up on the food-intake record almost immediately. In this way, more or less aside from the activity experiments, a considerable quantity of data was collected on age and sex differences in daily food-intake.

In the aforementioned experiments, each animal was placed in an activity cage. This cage which has been described in detail in a previous paper (2), consists of a revolving drum and a small living compartment. The revolving drum is connected by a series of levers to a ratchet cyclometer which automatically registers the number of turns of the drum clockwise and counter-clockwise. Thus the daily readings of the cyclometer serves as a rough but fairly accurate measurement of a rat's daily activity. The arrangement of the cage gives the animal free and easy access to food and water at all times.

Throughout all the work the external conditions were kept as uniform as possible. Constant dimmed illumination was maintained and a fairly uniform temperature (around 20°C.). The room in which the experiments were carried out was kept as quiet, well-ventilated and clean as possible. The room and all the cages were cleaned once every week—at 2:00 to 3:00 p.m. on Monday, and at this time all the animals were weighed. At noon every day the cyclometers were read and the food cups weighed. These gave the daily records of activity and food-intake. The food used was a synthetic diet recommended by Dr. E. V. McCollum, which gives very good results.

It was prepared according to the following formula:

	per cent
Whole wheat flour	72.5
Casein	10.0
Skimmed milk powder	10.0
Butterfat	5.0
Calcium carbonate ($CaCO_3$)	1.5
Sodium chloride (NaCl)	1.0

To each animal was given 70 grams of this food. As this quantity filled just half of a food cup the animal was not able to spill any food on the floor of the cage. Every day each food cup was replenished to this amount after weighing. Thus the animal had the same difficulty in getting food from day to day. The food in the cup was thoroughly stirred after each weighing to get a homogeneous mixture, so that the animal got the same kind of food daily. The water bottles were also refilled every day. If any food was found spilled on the floor of the cage or if there was any reason to suspect the food-intake record of being unreliable (for instance, when urine got into the food-cup, etc.) that day's record was discarded.

The individual food-intake curve of a female rat together with its body weight curve is given in figure 1a. The amount of daily food-intake and the weekly body weight, both in grams, are plotted on the ordinates, while the age of the animal is given on the abscissae. It is evident from these individual curves: *a*, that the amount of daily food-intake increases parri passu with the body weight during the period from twenty to thirty days after birth; *b*, that from thirty to fifty days after birth there is only a slight increase in the daily food-intake; and *c*, that after sixty days from birth the level of the food-intake curve remains constant, while the body weight continues to increase.

An average daily food-intake chart for twenty-nine female rats is presented in figure 1b and for seventeen male rats in figure 1c. These rats were used in different experiments, were of different age when the experiments were started, and were experimented with for different lengths of time. Therefore, the average daily food-intake charts for these rats were constructed in the following way. The weekly average of daily food-intake was first calculated for each of the rats and the individual values plotted by dots on the graph according to the age of the animals at the end of the respective weeks. These charts seem to show two phases; *a*, a gradual increase during the period from twenty to fifty days after birth, and *b*, a long plateau from fifty to two hundred and twenty days. In other words, there is phase I, before fifty days after birth, when the daily food-intake increases with age, followed by phase II, during which the food-intake remains constant (14 grams per day for the females and 15 grams for the males).

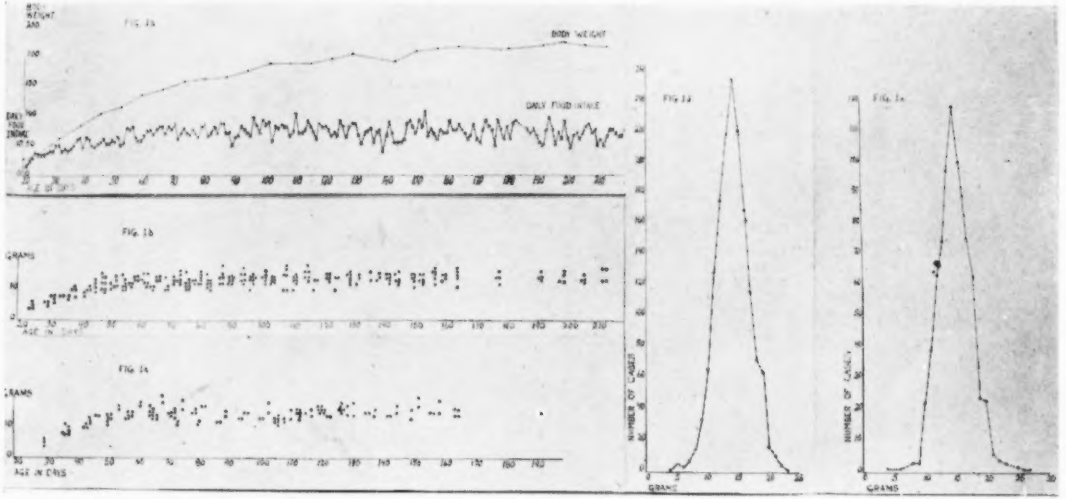


Fig. 1a. Food-intake and body-weight curves showing: *a*, the parri passu increase of daily food-intake with body-weight during the period from twenty to thirty days after birth; *b*, the slight increase in food-intake from thirty to fifty days after birth; *c*, the constant level of food-intake after sixty days from birth with continued increase in body-weight. Abscissae: age in days; ordinates: grams (inside scale—body-weight; outside scale—daily food-intake).

Fig. 1b. Average daily food-intake curve for female rats showing two phases: phase I, a gradual increase during the period from twenty to fifty days after birth; and phase II, a long plateau from fifty to two hundred and twenty days. Curve constructed from the data obtained from twenty-nine female rats. The dots represent the weekly average for daily food-intake for individual rats. Abscissae: age in days; ordinates: amount of food-intake.

Fig. 1c. Average daily food-intake curve for male rats, also showing the two phases: phase I, however, being of longer duration than in figure 1b. Curve constructed from data obtained from seventeen male rats. The dots represent the weekly average of daily food-intake of individual rats. Abscissae: age in days; ordinates: amount of food-intake.

Fig. 1d. Frequency curve of the daily food-intake of twenty-nine female rats observed twenty to two hundred and twenty days after birth. Abscissae: daily food-intake in grams; ordinates: number of cases; mean: 14 grams; median: 13.5 grams.

Fig. 1e. Frequency curve of daily food-intake of seventeen male rats. A comparison of this curve with that of figure 4 shows that the average daily food consumption of the male is greater than that of the female. Abscissae: daily food-intake in grams; ordinates: number of cases. Mean: 15.02 grams; median: 14.45 grams.

The constancy of the amount of the daily food-intake from fifty to two hundred and twenty days after birth can also be shown in another way. Frequency curves of the daily amount of food-intake for all the twenty-nine female rats and for the seventeen male rats experimented with from fifty to two hundred and twenty days after birth are presented in figures 1d and 1e. The curve for the females (fig. 1d) shows that the mode is 14 grams with a range of deviation from 4 to 23 grams. The median of this distribution curve is 13.5 grams. And the average daily food-intake of these female rats for this period is, as mentioned above, 14 grams. Since the mode, the median and the average agree so closely, it seems to be justifiable to say that a female rat from fifty to two hundred and twenty days old eats on the average 14 grams of McCollum's diet per day. Similarly, the curve for the males (fig. 1e) has the mode at 15 grams, while the median is at 14.45, and the average is 14.02, showing that the food-intake for the males is consistently 1 gram higher than for the females.

As the animals grow larger with age but with a constant daily food-intake, it will be expected that the need for food decreases with age. This is brought out by the graphs in figures 2a and 2b. In these graphs the weekly averages of daily food-intake for each rat were calculated as calories per kilogram of body weight.

$$\frac{F \times 1000}{\frac{W_1 + W_2}{2}} = C$$

F is the weekly average of daily food-intake in calories, W_1 the body weight of the animal taken on Monday of the week, W_2 the body weight of the animal taken on Monday of the following week and C the weekly average of daily food-intake as calories per kilogram of body weight. After the individual values were obtained, they were plotted by dots on the graph against age in days. These graphs also show clearly the two phases: *a*, a sharp fall from twenty to fifty days, and *b*, a very gradual drop thereafter.

When the weekly average of daily food-intake is represented as calories per square meter of body surface the two phases are still clearly apparent but are less distinct, and show greater individual variation than in the graph computed on body weight. The differences between the two methods of representation seem to be due to the method of calculating the body surface from the body weight. In this calculation the following formula (Meeh's) was used:

$$S = K\sqrt[3]{W^2}$$

S is the body surface in square meters, W the body weight in kilograms, and K a constant. According to Rubner, the constant for rats is 9.1. We

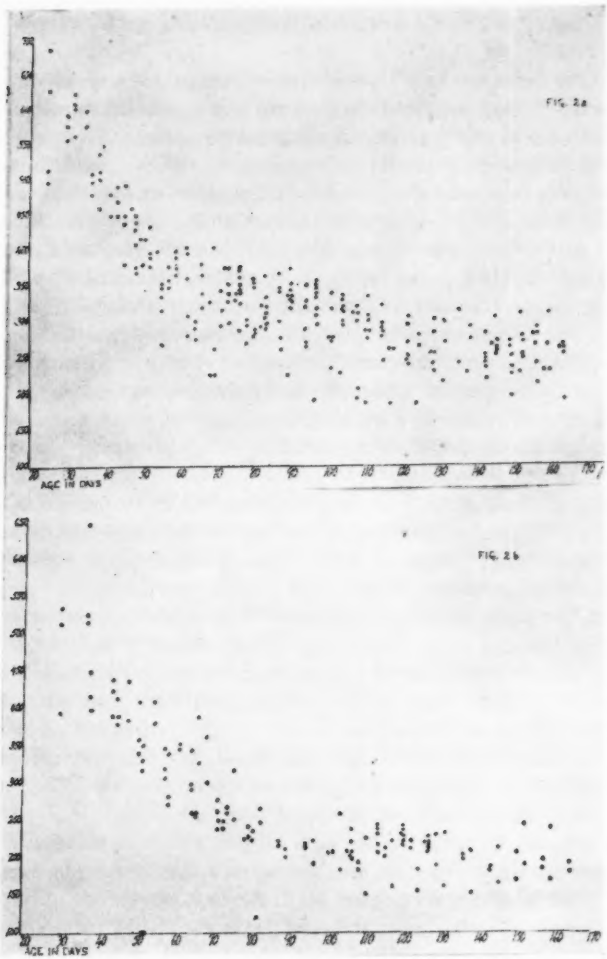


Fig. 2a. Graph showing the changes in need for nutrition with age in female rats. This graph also shows clearly two phases: *a*, a sharp fall from twenty to fifty days after birth, and *b*, a very gradual drop thereafter.

Data from twenty-nine female rats. The dots represent weekly averages of daily food-intake as calories per kilogram of body-weight for individual rats. Abscissae: age in days; ordinates: food-intake, in calories per kilogram of body-weight.

Fig. 2b. Same as figure 2a for male rats. A comparison of this graph with figure 2a shows that phase I is of longer duration in the curve for the males, and also that the level of phase II is lower for males than for females, that is, that the need for nutrition in the males (calculated as calories per kilogram of body-weight) is lower than in the females.

Data from seventeen male rats. Abscissae: age in days; ordinates: food-intake, in calories per kilogram of body-weight.

know that for humans the above-mentioned formula is not reliable. Also the value for K has not been verified by other investigators. It seems highly desirable to make some actual measurement of the body surface of rats of different weights and different ages. With a new formula the two methods of representation might show greater correspondence.

In all of these graphs, (figs. 1b, 1c, 1d, 1e, 2a, 2b) a sex difference is evident. After the animals reach phase II, the need for nutrition in the male rats is lower than in the females. A comparison of figures 2a and 2b shows this. Also, the outset of phase II is later in the males than in the females. This is evident when food-intake is considered in relation to either body weight or surface area. It shows clearly in figures 2a and 2b (based on body weight) in which the curve for the female rats reaches its final level about twenty days before that of the male rats.

To sum up, the observations reported in this paper are:

1. The average daily food-intake curve of the white rat shows two phases: *a*, a gradual increase during the period from twenty to fifty days after birth; and *b*, a constant level of food-intake from fifty to two hundred and twenty days, and probably from then on throughout the rest of the life of the rat, at least until senescence.

2. When the daily food-intake is calculated either as calories per kilogram of body weight or as calories per square meter of body surface, and graphs are made, they also show two phases: *a*, a sharp decrease during the period from twenty to sixty or ninety days after birth (according to sex), and *b*, a slow decrease thereafter.

3. The average daily food-intake curves of male and female rats run together up to fifty days after birth; from then on the curve for the males is about one gram higher than that for the females.

4. The graphs of average daily food-intake, either as calories per kilogram of body weight or as calories per square meter of body surface for both male and female rats run parallel in the first phase, but in the second phase the need for nutrition in male rats is lower than in female rats.

5. The graph of average daily food-intake as calories per kilogram of body weight or per square meter of body surface for male rats also differs in the beginning of the second phase from the same graph for female rats. In males the second phase begins about ninety days after birth, while in females it commences about sixty days after birth.

As pointed out in the beginning of this paper, these curves and graphs, obtained under as rigid and extensive control of environmental conditions as possible, may be regarded as representing what a rat would "spontaneously" eat at different ages. The explanation of these curves and graphs must then be sought in the changes within the organism.

The organism's need for energy is determined by the growth, the wear and tear, and the muscular and glandular activity of the organism.

Growth, maintenance and activity are all accompanied by chemical changes in the organism. As a result, some substances are taken in and some substances are given off by the cells. Since the blood is the chief carrier of nutrition and remover of waste products in the body, the chemical changes in the cells as consequences of growth, wear and tear and activity will produce changes in the constituents of the blood. And it seems to be these changes in the blood which produce "hunger" contractions in the stomach. (Carlson's experiments suggest this very strongly.) The "hunger" contractions will make the animal restless, seeking for food. When food is found and ingested, the animal will become quiet until it is again stimulated by gastric "hunger" contractions or by some other stimulation. The frequency of the periods of gastric "hunger" contractions will determine the times at which an organism will eat. The intensity of "hunger" contractions may determine the quantity of food an animal eats at each feeding period (provided plenty of food is accessible). Therefore, when kept under careful control and with ample food, the amount of food an animal will take per day is determined by the frequency and intensity of "hunger" contractions of the stomach; and in turn, the frequency and intensity of "hunger" contractions of the stomach are determined by the need of nutrition of the organism caused by growth, wear and tear, and muscular and glandular activities.

Looking at the food curves given in this paper from this point of view, it may be suggested that the first phase in the food-intake curve is mainly determined by the growth of the animal, while the second is determined by a balance between growth, wear and tear, and muscular and glandular activities. The sex differences of the food-intake curves may be similarly explained. In the male the longer first phase of the graph of daily food-intake as calories per kilogram of body weight may be due to the later maturity of males, while the lower second phase is very probably due to the fact that the male rat shows generally much less "spontaneous" muscular activity than the female.

BIBLIOGRAPHY

(1) Lusk: The elements of the science of nutrition. 3rd ed. 1921, 118–119.
(2) Wang: The relation between "spontaneous" activity and oestrous cycle in the white rat. Comp. Psychol. Monographs, 1923, ii, no. 6.

THE CHANGES IN THE AMOUNT OF DAILY FOOD-INTAKE OF THE ALBINO RAT DURING PREGNANCY AND LACTATION

GING H. WANG

From the Psychological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore

Received for publication October 22, 1924

In a previous paper we reported some observations on the daily food-intake of the albino rat during gestation and lactation. The food was the same as that described in the previous paper, a synthetic diet recommended by Dr. E. V. McCollum. These observations were made incidentally to some behavior experiments on the relation of spontaneous activity to the oestrous cycle. Since then a paper by John and Schick (1) has appeared on food-intake during gestation and lactation which stimulated us to re-examine our own data. This reëxamination has brought out a number of facts which entirely confirm the findings of John and Schick.

Our animals were kept in the regular cages that we use for activity experiments. These cages consist of a revolving drum with a small living compartment attached, as has been described in previous papers. It is important to emphasize, in connection with the present observations on food-intake, that only one animal was kept in each cage. The food eaten by the animal for twenty-four hours was measured every day at the same time. Food was left in the food-box where the rat had easy access to it at all times. It is important to repeat here also that the activity records taken on these animals did not in any way interfere with their food-intake.

In the activity experiments care was taken to determine the exact time of conception. This was done by mating the female only on the day of oestrus which was determined both by the smear method and by the activity record, the female being most active just before ovulation. After mating, each animal was examined for the "vaginal plug" which proved in all cases to be a definite sign of insemination.

Four rats were used in the experiments, S1, S3, S4, S5. S4 was inseminated on the 100th day after birth, S3 on the 109th day, S5 on the 111th day and S1 on the 112th day. The gestation period was twenty-three days in length for S5 and twenty-two days for the other three rats. There were ten young in each litter but only that of S3 was raised without loss. One of S5's litter and two each of the litters of S1 and S4 died of

asphyxiation. (This was because the living cage was too small for breeding purposes.) All the litters were weaned on the twenty-first day after birth. No attempt was made to weigh the young. Also some of the mothers which became quite excitable after parturition were not weighed during either the first or second week after the litters were cast.

In figure 1a are given the daily food-intake and the weekly body weight curves of S5 (the food-intake curves for S3 and S4 were given in the previous paper). From these curves it is evident: *a*, that there is no considerable increase in the daily amount of food-intake during the gestation period, though there is marked increase in body weight; *b*, that the increase in the daily food-intake starts right after the delivery and increases very rapidly; *c*, that there is a sharp drop in the level of the food-intake curve after weaning; and *d*, that the level of the food-intake curve is slightly higher after lactation than before conception.

These findings are confirmed when a comparison is made between the average amounts of daily food-intake for the twenty days before concep-

TABLE 1

Showing increase in per cent in amount of food-intake over average

NUMBER OF RAT	PERCENTAGE INCREASE DURING GESTATION	PERCENTAGE INCREASE DURING LACTATION	PERCENTAGE INCREASE AFTER LACTATION
S1	5.28	142.32	16.69
S3	13.53	141.26	6.32
S4	11.16	138.26	8.55
S5	0.87	113.57	1.46
Averages.....	7.71	133.85	8.20

tion, for the twenty-two to twenty-three days of gestation, for the twenty-one days of nursing and for the twenty days after weaning. (See fig. 1b.) The increase during pregnancy is quite slight (about 1 gram) and just about the same as the increase after weaning. But the increase during lactation is enormous. Table 1 gives the percentage of increase in food-intake during gestation, and during and after nursing based on the average for the twenty days before pregnancy.

However, the increase in the average daily amount of food-intake during gestation and after lactation is only apparent. If we take the average daily amount of food-intake as calories per kilogram of body weight rather than at its face value, we find that there is a decrease instead of an increase during both the period of pregnancy and the twenty days after nursing. This is clearly shown in figure 1c. The same is true when the average daily food-intake is computed as calories per square meter of body surface.

The decrease in the amount of food-intake after lactation is due to the fact that the animals are growing larger with age. As will be shown in

another paper, after maturity, the older the rat, the less the average daily amount of food-intake either as calories per square meter of body surface or as calories per kilogram of body weight. And the decrease of food-intake after lactation in all these four animals experimented on comes very close to the average food-intake as calories either per kilogram of body weight or square meter of body surface for female rats of the same age.

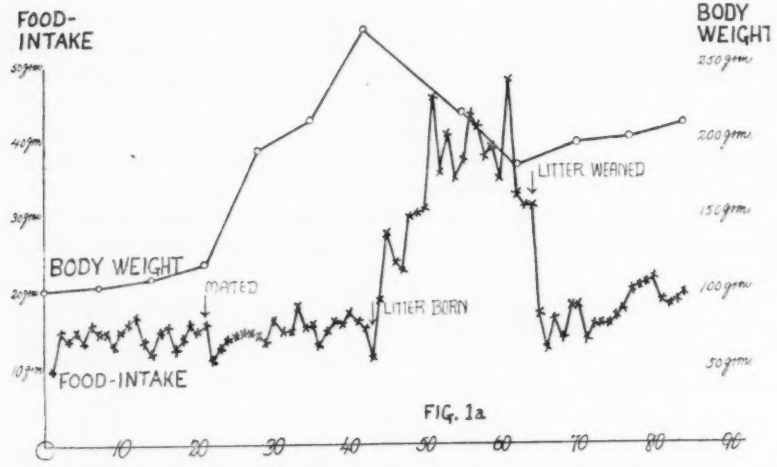


Fig. 1a. Curves showing the change in daily food-intake and weekly body-weight during gestation and lactation.

Rat S5. Age 92–175 days.

Ordinates: Course of experiment in days.

Abscissae { Food-intake in grams for the food-intake curve. / Body-weight in grams for the body-weight curve.

The curve shows:

a. Almost no increase in daily food-intake during the gestation period, but marked increase in body weight.

b. A rise in daily food-intake appearing immediately after delivery and increasing rapidly.

c. A sharp drop in the food-intake curve after weaning.

d. A slightly higher level of the food-intake curve after lactation than before conception.

But the decrease in food-intake during the gestation period seems strange. It seems probable that the need of nourishment for supporting the growth of embryos causes an increase in the food-intake of the mother. Schick suggests that human individuals instinctively refrain from bodily activity during pregnancy and that this covers up the increased demand for nourishment. This appears also to be true with the rat. There is, as shown in the previous paper, a big decrease in activity after conception.

The nearer to the day of delivery, the greater the decrease. The average decrease in daily activity during the gestation period calculated as percentage of the average amount of daily activity for the twenty days before insemination, is about 90 per cent in each of the four animals experimented on. It is interesting to note as shown in table 2 that the animal showing

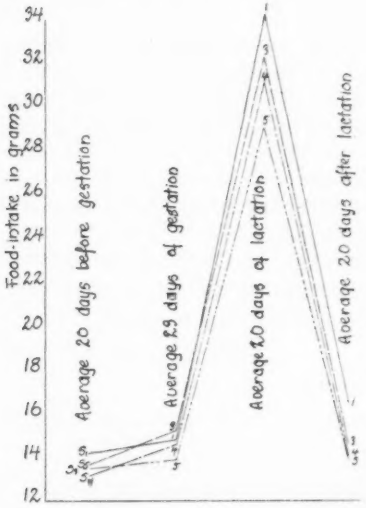


Fig. 1b

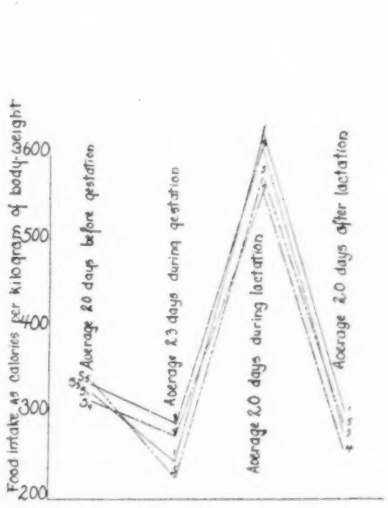


Fig. 1c

Fig. 1b. Curves showing the changes in average daily food-intake before and during gestation and during and after lactation for four rats: S1, S3, S4, S5.

Ordinates: 1, average of 20 days before conception; 2, average of the period of gestation; 3, average of the period of nursing; 4, average of 20 days after weaning.

Abscissae: Food-intake in grams.

The increase in daily food-intake during pregnancy is very slight,—while the increase during lactation is enormous. When food-intake is computed as calories per kilogram of body weight (fig. 1c) or as calories per square meter of body surface the apparent increase during the gestation period appears as a decrease.

Fig. 1c. Same data as figure 1b, expressed as calories per kilogram of body-weight.

Ordinates: as in figure 1b.

Abscissae: Calories per kilogram of body-weight. The decrease after lactation is due to the fact that the animals are growing larger with age.

the greatest decrease in daily activity is the animal showing the least increase in food-intake. Thus for each animal the decrease in activity is in inverse proportion to the increase in food-intake. The energy from food which may be spent in muscular activity is now presumably used up in the building up of tissues.

The decrease in daily activity perhaps also plays a part in adapting the animal to the need of nourishment during the period of lactation. Here the decrease in daily activity (calculated as percentage of the average daily activity of the twenty days before pregnancy) is about 85 per cent. The relation between decrease in daily activity and increase in food-intake during lactation is the same as the relationship during pregnancy, as the figures in table 3 show.

To sum up, the facts obtained from the experiments are: *1*, during gestation there is no increase in food-intake; *2*, there is an enormous increase in food-intake after parturition; *3*, there is a quick return to normal level after weaning; *4*, there is a marked decrease in daily activity during

TABLE 2

Showing relation between average daily food-intake and activity during pregnancy

NUMBER OF RAT	AVERAGE DAILY FOOD-INTAKE AS CALORIES PER KILOGRAM OF BODY WEIGHT	DECREASE IN DAILY ACTIVITY
		per cent
S3	291	84
S4	272	90
S1	245	92
S5	226	96

TABLE 3

Showing relation between average daily food-intake and activity during lactation

NUMBER OF RAT	AVERAGE DAILY FOOD-INTAKE AS CALORIES PER KILOGRAM OF BODY WEIGHT	DECREASE IN DAILY ACTIVITY
		per cent
S1	630	83
S3	610	85
S5	576	88
S4	559	93

both pregnancy and lactation; and *5*, the decrease in daily activity seems to be inversely proportional to the changes in daily food-intake.

The first three facts agree with the findings of John and Schick, while the last two bear out their contention. With a much larger series of experiments, John and Schick have also shown that the increase in daily food-intake during lactation is in proportion to the number of young and parallels the increase in body-weight of the new-borns.

As pointed out in a previous paper, the adult rats have a constant level of daily food-intake. This constant level seems to be determined by the balance between growth, wear and tear, and muscular and glandular activity of the organism. Then any change in the equilibrium of this

balance will bring about readjustment between these three factors and the food-intake. What happens during gravidity is probably a readjustment between growth and muscular activity. We know that there is a 90 per cent decrease in activity. The energy saved by this decrease is very probably used for the growth of the embryos and their adnexa. This readjustment between muscular activity and growth makes it unnecessary to have an increase in daily food-intake. After parturition, the activity level still remains low. But the rapid growth of the young makes greater demands than can be met by the decrease in muscular activity. The bigger the young, the more milk they suck. The milk production must keep pace with the growth of the young. The increased activity of the mammary glands then causes changes in the blood constituents, which increase either or both the frequency and the intensity of the "hunger" contractions of the stomach. The change in the frequency and intensity of "hunger" contractions will bring about a change in the daily food-intake. Besides this, it seems that the increased activity of the mammary glands may produce also other physiological changes in the gastro-intestinal tract which enable the animal to utilize three times the normal amount of food-intake.

BIBLIOGRAPHY

(1) JOHN UND SCHICK: Zeitschr. f. Kinderheilk., 1923, xxxiv, 239.
(2) WANG: The relation between the "spontaneous" activity and oestrous cycle in the white rat. Comp. Psychol. Monographs, 1923, ii, no. 6.
(3) WANG: This Journal, 1925, lxxi, 729.

THE AMERICAN JOURNAL OF PHYSIOLOGY is issued monthly by the American Physiological Society under the direction of the Council of the Society. From three to four volumes, each of about six hundred and fifty pages, are published yearly. The subscription price per volume in the United States and Canada is $5.00; in other countries, $5.25.

Manuscripts submitted for publication may be sent to any member of the Council or to the Managing Editor, Dr. D. R. Hooker, 19 W. Chase Street, Baltimore, Md. They should be **packed flat, carefully protected from injury, and insured.**

Each article should conclude with a **brief summary of results** suited to the needs of reference journals.

References to literature must accord with the following arrangement. **In the text** the author's name is to be followed by the year date of the publication in parenthesis, thus Smith (1911). If the author's name is not mentioned in the text, the parenthesis is to contain that name as well as the date, thus (Smith, 1911). If reference is made to more than one publication of an author in a given year, the year date is to be followed by the letters a, b, c, etc., thus Smith (1911a). **The bibliography** at the end of the article is to list all text references according to authors' names, with initials, alphabetically arranged, thus, Smith, J. C. 1911. Journ. Biol. Chem., xii, 306.

All figures for illustration must be submitted in such form as to admit of photographic reproduction without retouching or redrawing. Marginal letters or figures cannot be set in type and must, therefore, be drawn in India ink by the author and regarded as part of the figure. Unless specific instructions are given by authors, the printer will be allowed to use his judgment in determining the amount of reduction on all figures. The JOURNAL cannot accept illustrations which, when reproduced for printing, exceed the size of a JOURNAL page ($4\frac{1}{2} \times 7\frac{1}{2}$ inches), unless the author is prepared to pay the cost of an insert page.

Plotted curves must be drawn in India ink on **blue lined coördinate paper.** Blue ink will not show in reproduction, so that all guide lines, letters, etc., must be in India ink.
